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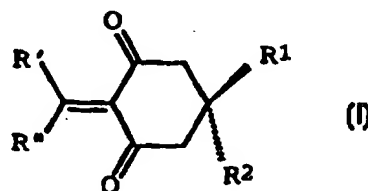
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<p>(21) International Application Number: PCT/AU97/00544  (22) International Filing Date: 26 August 1997 (26.08.97)  (30) Priority Data: PO 1905 26 August 1996 (26.08.96) AU  (71) Applicant (for all designated States except US): ALCHEMIA PTY. LTD. [AU/AU]; Suite 4, 7 Primrose Street, Sherwood, QLD 4075 (AU).  (72) Inventors; and (75) Inventors/Applicants (for US only): TOTH, Istvan [GB/GB]; 195 Church Road, Northolt, Middlesex UB5 5BE (GB). DEKANY, Gyula [HU/GB]; 157 Heriway Avenue, Ruislip, Middlesex HA4 6HS (GB). KELLAM, Barry [GB/GB]; 93 Poplar Grove, Maidstone, Kent ME16 0AL (GB).  (74) Agent: SANTER, Vivien, B.; Griffith Hack, 509 St. Kilda Road, Melbourne, VIC 3004 (AU).</p>	<p>(81) Designated States: AU, CA, CN, HU, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.</p>	

(54) Title: OLIGOSACCHARIDE SYNTHESIS

(57) Abstract

The invention provides a system for solid-phase synthesis of oligosaccharides, based on the discovery that a 2-substituted-1,3-dioxocycloalkyl linker group of general formula (I) can be used to couple saccharide groups of both the O-glycoside and N-glycoside type to a polymer support. The invention provides reagents, reagent kits and methods for solid-phase oligosaccharide synthesis.



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OLIGOSACCHARIDE SYNTHESISFIELD OF THE INVENTION

This invention relates to methods for synthesis  
5 of oligosaccharides, and in particular to methods for solid  
phase or combinatorial synthesis of oligosaccharides. The  
invention provides a novel linker-resin, linker-saccharide,  
or resin-linker-saccharide complex, which in one embodiment  
10 enables a saccharide residue to be linked to a soluble or  
insoluble polymeric support for use as a basis for solid-  
phase synthesis of oligosaccharides. In a second  
embodiment, the complex of the invention enables  
oligosaccharides to be linked to a solid polymeric support  
for use as an analytical reagent.

15

BACKGROUND OF THE INVENTION

Oligosaccharides constitute a major class of  
bioactive polymers, implicated in biochemical processes  
(Lasky, 1992; Varki, 1993) as diverse as cellular  
20 differentiation, hormone-cell recognition and cell-cell  
adhesion, especially viral-host cell (Gambaryan et al,  
1995) and bacteria-host cell attachment (Boren et al,  
1993). Involvement of oligosaccharides in diseases such as  
cancer, cardiovascular disorders, microbial infections,  
25 graft rejection and autoimmune disorders has therefore,  
been strongly suggested. Conjugation of carbohydrates to  
bioactive peptides has also been demonstrated to stabilise  
the peptides against degradation, and, in more specific  
circumstances, to facilitate peptide transport across  
30 biological barriers (Lee, 1989; Fisher, 1991; Rodriguez,  
1989). Thus the ability to synthesise oligosaccharides in  
a facile and efficient manner is now becoming an extremely  
important area within organic chemistry.

The highly labour intensive solution phase  
35 strategies hitherto utilised in oligosaccharide syntheses  
require an extremely specialised knowledge and a high  
degree of chemical skill. This situation was mirrored

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within the area of peptide synthesis, until Merrifield et al proposed and developed Solid Phase Peptide Synthesis (SPPS) over thirty years ago (Merrifield, 1963). In SPPS immobilisation of the first amino acid of the required sequence to an insoluble resin enabled large excesses of reagents to be used to achieve the coupling of the second amino acid. Any unused materials remaining at the end of the coupling step could then be removed simply by washing the resin beads. This technology meant that the chemist could drive each coupling reaction to almost quantitative yields, and since the peptide intermediates formed were still bound to the resin, purification after each acylation step was not required. SPPS enables peptide and polypeptide synthesis to be employed as a routine research and synthetic tool, and permits large-scale combinatorial synthesis of peptides for screening of potential pharmaceutical agents.

For many years chemists have attempted to transpose this solid-phase methodology to oligosaccharide synthesis, with varying degrees of success. The first attempt was approximately 25 years ago (Frechet and Schuerch, 1971; Frechet and Schuerch, 1972; Guthrie et al, 1971; Guthrie et al, 1973). However, the ozone-mediated deprotection product was an aldehyde-substituted glycoside. Danishefsky and coworkers described the solid phase synthesis of the Lewis b Antigen (Randolph et al, 1995) and N-linked glycopeptides (Roberge et al, 1995) by initial attachment of the primary sugar unit of the oligosaccharide to a 1% divinylbenzene-styrene co-polymer support via a silyl ether linkage. The resin-bound sugar moiety was in this instance a glycal, with on-resin activation achieved via epoxidation of the double bond, and the resulting glycal residue acting as a sugar donor through nucleophile ring-opening of the epoxide. Since there are no colorimetric methods available to the sugar chemist to monitor on-resin glycosylations, the only means of assessing the progress of the reaction is by lysis of the

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oligosaccharide-resin bond and subsequent analysis of the cleavage product, usually by thin layer chromatography. The tetra-n-butylammonium fluoride-mediated deprotection conditions required to cleave Danishefsky's silyl ether linker are both hazardous and slow. This coupled with the requirement for on-resin activation of the tethered glycals, makes the overall strategy and methodology far from ideal.

In an alternative approach, Douglas and coworkers described the synthesis of D-mannopentose using a polyethyleneglycol  $\omega$ -monomethylether co-polymer and a succinoyl or an  $\alpha,\alpha'$ -dioxxylyl diether linker (Douglas et al, 1995). The reactions were carried out in solution phase, with removal of unused reactants being achieved by precipitation of the oligosaccharide-polymer complex and subsequent washing. In the latter example, cleavage of the oligosaccharide-polymer bond was achieved through catalytic hydrogenation, which required exposure of the conjugate to 1 atm of H<sub>2</sub> for 48 h to achieve respectable yields. This again is far too slow to allow effective monitoring of individual glycosylation reactions. Yan et al reported sulphoxide-mediated glycosylation on a Merrifield resin, using a thiophenol linker for the attachment of the primary sugar residue (Yan et al, 1994). This method resulted in the construction of (1-6)-linked oligosaccharides, and was suitable for synthesis of both  $\alpha$ - and  $\beta$ -glycosidic linkages. However, the thioglycosidic linkage to the resin dictates that similar sugar donors cannot be employed in this strategy.

Recently Rademann and Schmidt reported the use of trichloroacetimidate sugar donors to a resin bound sugar tethered via an alkyl thiol (Rademann and Schmidt, 1996); once again, however, this method precludes the use of the far superior thioglycoside sugar donors. Meanwhile, Adinolfi et al described the synthesis of disaccharides using a polyethyleneglycol-polystyrene resin, with connection of the first sugar to the polymeric support

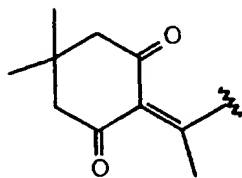
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through a succinate spacer (Adinolfi et al, 1996). However, the acid lability displayed by this linker means that the primary sugar cannot be linked to the resin via the glycosidic position.

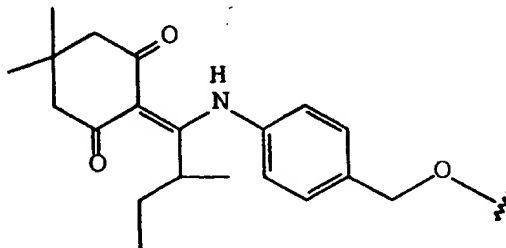
5           The above examples serve to illustrate that the critical element in solid phase synthesis is the nature of the linker between the solid support and the initial synthon. The linker must display excellent stability to the conditions of coupling and deprotection, yet in the case of solid phase oligosaccharide synthesis, it should also be rapidly and efficiently cleaved to allow monitoring of the progress of individual coupling reactions. The cleavage should ideally be achieved by the use of a relatively innocuous chemical reagent.

15           It is clear, then, that there remains a need in the art for simple, efficient and economical methods for solid-phase synthesis of oligosaccharides.

          A hydrazine-labile primary amino-protecting group, *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde), has been reported for protection of lysine side chains during SPPS (Bycroft et al, 1993). This group was modified for use as a carboxy-protecting group in SPPS when the 2-(3-methylbutyryl)dimedone analogue of 2-acetyl-dimedone was condensed with 4-aminobenzylalcohol to afford 4-[*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino]benzyl ester (ODmab) (Chan et al, 1995).



Dde



ODmab

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The two protecting groups were reported to be stable to the deprotecting conditions widely used in SPPS, ie. trifluoroacetic acid (TFA) or 20% piperidine in dimethyl formamide (DMF). The ethyl ester, 4-[N-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl)amino]benzyl ester (ODab) showed small but significant instability to 20% piperidine-DMF. Both Dde and ODmab are linked to groups on amino acids, rather than directly to the solid-phase support. Their use in solid-phase oligosaccharide synthesis has not been suggested.

We have now surprisingly found that protecting groups similar to Dde and ODmab can be coupled to a polymeric support, thereby generating a system for the immobilisation of sugars. To this end we have immobilised N- and O-glycosides to the solid support and synthesised oligosaccharides using various sugar donors. The linkers display excellent stability to most acids and secondary/tertiary bases encountered in modern synthetic chemistry, yet are rapidly and efficiently cleaved with either ammonia, hydrazine or primary amines.

Bannwarth *et al* have independently developed a different solid phase linker around the Dde protecting group, which they have utilised for the immobilisation of amino acids and primary amines for combinatorial library synthesis (Bannwarth *et al*, 1996). However, the synthesis of this linker is both protracted and inefficient, and the linker only displays a limited stability to secondary bases such as piperidine. There has been no suggestion that this linker could be used for oligosaccharide synthesis. The linkers we have developed demonstrate a far greater stability than those of Bannwarth *et al*.

#### SUMMARY OF THE INVENTION

In one aspect, the invention provides a support for solid-phase synthesis of oligosaccharides, said support comprising:

a) a resin,

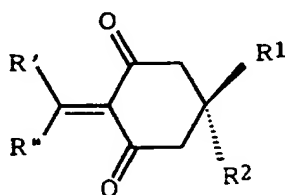
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b) a linker covalently attached to the resin,  
and

c) one or more saccharide groups covalently  
attached to the resin via the linker,

5 wherein the linker is a 2-substituted-1,3-  
dioxocycloalkane compound, and

said support having general formula I:



10

I

in which

15  $R^1$  and  $R^2$  may be the same or different, and is  
each hydrogen or  $C_{1-4}$  alkyl;

$R'$  is an amino sugar, a glycosylamine, or a  
glycosylamine of an oligosaccharide; a mono or  
oligosaccharide coupled through an alkyl-, substituted  
alkyl-, aryl-, substituted aryl-, cycloalkyl-, or  
20 substituted cycloalkyl-amino group; or a mono or  
oligosaccharide coupled through a carboxyalkyl-,  
substituted carboxyalkyl-, carboxyaryl-, substituted  
carboxyaryl-, carboxycycloalkyl-, or substituted  
carboxycycloalkyl-amino group; and

25  $R''$  is an alkyl, substituted alkyl, aryl,  
substituted aryl, cycloalkyl, or substituted cycloalkyl  
spacer group which is directly coupled to the resin  
support, or which may optionally be coupled to the resin  
support via a suitable covalent linkage, which is stable to  
30 conditions of oligosaccharide synthesis and cleavage.

The covalent linkage to the resin may suitably be  
provided by a -CONH-, -O-, -S-, -COO-, -CH=N-, -NHCONH-,  
-NHCSNH, or -NHNH- grouping, eg. Spacer-CONH-resin, Spacer-

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O-resin, Spacer-S-resin, Spacer-CO<sub>2</sub>-resin, Spacer-CH=N-resin, Spacer-NHCONH-resin, Spacer-NHCSNH-resin, Spacer-NHNH-resin. Other possible covalent linking groups will be known to those skilled in the art.

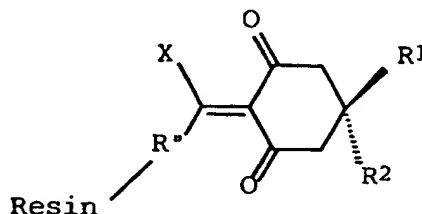
5 Preferably both R<sup>1</sup> and R<sup>2</sup> are methyl.

Preferably R' is an oligosaccharide-O-CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)-NH, monosaccharide-O-CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)-NH, amino-oligosaccharide-CO<sub>2</sub>CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)NH, or amino-monosaccharide-CO<sub>2</sub>CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)-NH group.

10 In a particularly preferred embodiment the 2-substituted-1,3-dioxocycloalkane linker is functionalised Dde, Ddh or ODmab. In one very particularly preferred embodiment the support comprises a resin, a linker and a monosaccharide, an oligosaccharide, an aminosaccharide or  
15 an amino-oligosaccharide.

In a second aspect, the invention provides a support for solid-phase synthesis comprising a resin and a linker group, wherein the linker is a 2-substituted-1,3-dioxocycloalkane of general formula II:

20



II

25 in which

X is OH or NH<sub>2</sub>;

R<sup>1</sup> and R<sup>2</sup> may be the same or different, and is each hydrogen or C<sub>1-4</sub> alkyl; preferably both R<sup>1</sup> and R<sup>2</sup> are methyl; and

30 R' is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group which is directly coupled to the resin

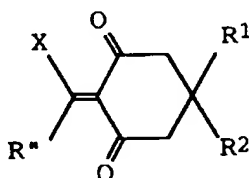
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support, or which may optionally be coupled to the resin support via a suitable covalent linkage, which is stable to conditions of oligosaccharide synthesis and cleavage. The covalent linkage may suitably be provided by a -CONH-, -O-,  
 5 -S-, -COO-, -CH=N-, -NHCONH-, -NHCSNH, or -NHNH- grouping, eg. Spacer-CONH-resin, Spacer-O-resin, Spacer-S-resin, Spacer-CO<sub>2</sub>-resin, Spacer-CH=N-resin, Spacer-NHCONH-resin, Spacer-NHCSNH-resin, Spacer-NHNH-resin. Other possible covalent linking groups will be known to those skilled in  
 10 the art.

In a third aspect, the invention provides a linker-saccharide complex, comprising a linker group of general formula II as defined above and a saccharide group as defined above for R'.

15 In a fourth aspect the invention provides a linker compound carrying functional groups suitable to attach a primary amine to a resin via covalent bonds which are stable to conditions of oligosaccharide synthesis and cleavage, said compound having general formula III

20



III

in which

X is OH or NH<sub>2</sub>;

25 R<sup>1</sup> and R<sup>2</sup> may be the same or different, and is each hydrogen or C<sub>1-4</sub> alkyl, and

R'' is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group, which carries a functionality capable of  
 30 reacting with a functionalised resin.

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Preferably the linker compound is 6-hydroxyl-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid or an ester thereof. Preferably the ester is a benzyl, methyl, or t-butyl ester.

5 For the purposes of this specification the term "substituted" in the definitions of substituents within this specification means that the substituent is itself substituted with a group which does not change the general chemical characteristics of the substituent. Preferred  
10 such further substituents are halogen, nitro, amino, hydroxyl, and thiol; preferred halogens are chlorine and iodine. The person skilled in the art will be aware of other suitable substituents of similar size and charge characteristics which could be used as alternatives in a  
15 given situation.

For the purposes of this specification a compound is regarded as "stable to conditions of oligosaccharide synthesis and cleavage" if there is less than 10% loss of the compound after exposure at room temperature to ammonia,  
20 hydrazine or a primary amino compound in water or DMF. The person skilled in the art will readily be able to determine whether the stability of a particular compound is adequate for it to be useful for the purposes of the invention, using conditions appropriate for his or her particular  
25 needs.

The linker compound of the invention may be synthesized on the resin, or may be synthesized in solution.

The invention also provides kits useful in solid  
30 phase synthesis or combinatorial synthesis of oligosaccharides, comprising either

- a) a resin-linker-saccharide support,
  - b) a linker-saccharide complex, or
  - c) a resin-linker support,
- 35 according to the invention, as described above. The kit may optionally also comprise one or more further reagents such as protecting agents, deprotecting agents, and/or

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solvents suitable for solid phase or combinatorial synthesis. The person skilled in the art will be aware of suitable further reagents. Different types of kit can then be chosen according to the desired use.

5           The resin may be any resin which swells in water and/or in an organic solvent, and which comprises one of the following substituents: halogen, hydroxy, carboxyl, SH, NH<sub>2</sub>, formyl, SO<sub>2</sub>NH<sub>2</sub>, or NHNH<sub>2</sub>, for example methylbenzhydrylamine (MBHA) resin, amino or carboxy  
10   tentagel resins, 4-sulphamylbenzyl AM resin. Other suitable resins will be known to those skilled in the art.

          The invention also provides a method of solid-phase synthesis of oligosaccharides, comprising the step of sequentially linking mono- or oligosaccharide groups to a  
15   support as described above. Similarly the mono- or oligosaccharide building blocks may be as described above.

          This method is particularly useful for combinatorial synthetic application.

          The linker compound may be synthesised in  
20   solution or directly on the resin in a stepwise manner prior to the coupling of the initial sugar group, or the linker-initial sugar conjugate may be synthesised in solution phase and subsequently coupled to the solid support, with subsequent sugars being sequentially  
25   attached. Preferably the second and all subsequent sugar groups are coupled to the oligosaccharide chain-resin conjugate after the last sugar in the oligosaccharide chain is partially deprotected.

          The invention accordingly provides a method of  
30   synthesis of a linker group according to general formula I as defined above, comprising the step of C-acylation of a 2-substituted 1,3-dioxocyclohexane compound with a dicarboxylic acid. Preferably the dicarboxylic acid is mono-protected by ester formation. More preferably the  
35   reaction is activated with carbodiimide and catalysed by N,N'-dimethylaminopyridine.

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The product of the reaction may optionally be reacted with 4-aminobenzyl alcohol, to form the 4-aminobenzyl derivative.

The invention also provides a method of synthesis  
5 of a resin-linker support, comprising the step of swelling a resin in a suitable solvent, treating the swollen resin with a dicarboxylic acid, and reacting the thus-produced product with a 2-substituted 1,3-dioxocycloalkane compound. Preferably for both synthesis of the linker and synthesis  
10 of the resin-linker support the 2-substituted 1,3-dioxocycloalkane compound is 5,5-dimethyl-1,3-cyclohexanedione. Also preferably the dicarboxylic acid is adipic acid.

The first sugars attached to the resin-linker  
15 unit may be unprotected, partially protected or fully protected glycosides, aminoglycosides, or ether- or amino-linked sugars, where the coupling takes place through a non-glycosidic position.

The building block mono- or oligosaccharide-  
20 donors may be any activated sugar, including but not limited to orthoesters, thioorthoesters, cyanoalkylidene derivatives, 1-O-acyl sugars, amino sugars, acetimidates, trichloroacetimidates, thioglycosides, aminoglycosides, amino-oligosaccharides, glycosylamines of oligosaccharides,  
25 glycosyl thiocyanates, pentenyl glycosides, pentenoylglycosides, isoprenyl glycosides, glycals, tetramethylphosphoro diamidates, sugar diazirines, selenoglycosides, phosphorodithioates, glycosyl-dialkylphosphites, glycosylsulphoxides and  
30 glycosylfluorides.

Preferably the first sugar coupled to the resin is an aminosugar, an aminoglycoside, or an amino-oligosaccharide or a glycosyl amine of an oligosaccharide.

Preferably partial sugar deprotection is achieved  
35 by using acyl-type, trityl, benzyl-type, acetal-type, or various silyl and/or photolabile protecting groups in addition to permanent protecting groups. This permits the

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synthesis of branched oligosaccharides by using two orthogonal hydroxy-protecting groups on a single sugar donor.

The synthesised oligosaccharide can be cleaved from the resin using ammonia, hydrazine or a primary amine, such as butylamine or cyclohexylamine. For the preparation of aminoglycosides, ammonia or a suitable primary amine in an organic solvent is preferably employed. For the preparation of hydrazides, hydrazine in water or in an organic solvent is preferably employed. For the preparation of oligosaccharides, ammonia in water or in an organic solvent is preferably employed, followed by acidification. When the linker contains a 4-aminobenzyl moiety, after cleavage as described above the first sugar is released still protected by the aminobenzyl group; this can be removed by hydrogenation if desired.

The person skilled in the art will appreciate that the oligosaccharide can be retained on the resin for use as an analytical or preparative reagent, for example in affinity chromatography or for bulk-scale affinity separation.

#### Detailed Description of the Figures

Figure 1 shows a general representation of the strategy required for solid phase oligosaccharide synthesis.

Figure 2 illustrates a general representation of the 'divide-couple-recombine' method of oligosaccharide library synthesis utilising a solid phase strategy.

Figure 3 shows the synthesis of the Dde-based linker of the invention, attachment of the primary sugar residue and coupling of the sugar-linker conjugate to a resin support. An alternative approach whereby the linker is synthesised directly on the resin is also shown.

Figure 4 shows the synthesis of the ODmab-based linker of the invention, attachment of the primary sugar

residue and coupling of the sugar-linker conjugate to the resin support.

Figure 5 shows the cleavage of the oligosaccharide-linker bond in a resin-bound hydrazine mediated deprotection product.

Figure 6 shows a general representation of the selective deprotection of one sugar hydroxyl group, and subsequent coupling of the next sugar donor.

Figure 7 shows the immobilisation of an amino-oligosaccharide on the Dde-derivatised support.

Figure 8 shows a list of activated sugar donors for solid-phase synthesis.

Figure 9 shows the synthesis of a differentially protected thioglycoside and a partially protected aminoglycoside.

Figure 10 shows the trichloroacetimidate activation of the 4-aminobenzyl modified linker.

Figure 11 shows ammonia-mediated cleavage of the aminoglycoside with post-cleavage acidification to generate the free carbohydrate.

Figure 12 shows a specific example of the general strategy for oligosaccharide synthesis employing a thioglycoside as the sugar donor.

Figure 13 shows another specific example of the general strategy for oligosaccharide synthesis employing a thioglycoside as the sugar donor.

Figure 14 shows the cleavage of a monosaccharide bound to the 4-aminobenzyl modified linker.

Figure 15 shows an example of a resin-bound fully protected trisaccharide.

Figure 16 shows the immobilisation of an unprotected amino sugar.

#### Detailed Description of the Invention

Abbreviations used herein are as follows:

Bn Benzyl

Bu Butyl

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	DCM	Dichloromethane
	Dde	N-1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl
	Ddh-OH	6-Hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)hexanoic acid
5	DMAP	N,N'-Dimethyl aminopyridine
	DMF	N,N'-Dimethylformamide
	DMTST	Dimethyl(methylthio)sulphonium trifluoromethanesulphonate
	EEDQ	1-Isobutyloxycarbonyl-2-isobutyloxy-1,2-
10		dihydroquinoline
	EtOAc	Ethyl acetate
	EtOH	Ethanol
	FAB-MS	Fast atom bombardment mass spectrometry
	HRMS	High resolution mass spectrometry
15	MBHA	Methyl benzyhydrilamine resin
	Me	Methyl
	MeOH	Methanol
	NMR	Nuclear magnetic resonance
	ODmab	4-(N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-
20		3-methylbutyl]-amino}benzyl alcohol.
	PEG	Polyethylene glycol
	tBu	tetra-butyl
	TFA	Trifluoroacetic acid
	THF	Tetrahydrofuran
25	TLC	Thin-layer chromatography
	TNBS	2,4,6-Trinitrobenzene sulphonic acid

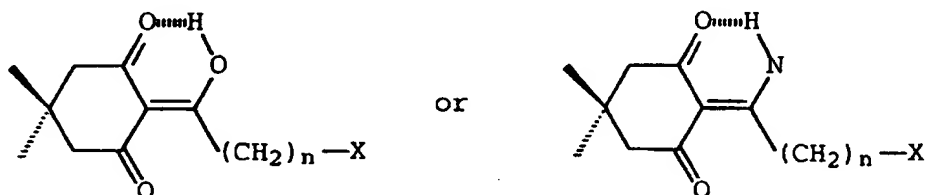
The invention is based upon the immobilisation of a Dde-, Ddh or ODmab-based linker to a polymer support in order to tether any saccharide or oligosaccharide group. This has been illustrated by the coupling of N- and O-glycosides to the linkers, which have been used for oligosaccharide synthesis following coupling to the resin. The nature of these linkers is such that as well as the potential to immobilise any type of sugar, any sugar donor can be subsequently used for oligosaccharide synthesis, thereby allowing rapid and efficient coupling procedures.

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Suitable sugar donors include, but are not limited to orthoesters, thioorthoesters, cyanoalkylidene derivatives, 1-O-acyl sugars, acetimidates, trichloroacetimidates, thioglycosides, glycosyl thiocyanates, pentenyl glycosides, pentenoylglycosides, isoprenyl glycosides, glycals, tetramethylphosphoro diamidates, sugar diazirines, selenoglycosides, phosphorodithioates, glycosyl-dialkylphosphites, glycosylsulphoxides and glycosylfluorides.

The stability of the linkers means that orthogonal hydroxy-protecting groups can be employed in sugar protection. These protecting groups include, but are not limited to, acyl-type, trityl, benzyl type, acetal type or various silyl and photolabile protecting groups.

The ease of linker synthesis means that the second functional group on the linker may be a halogen, alcohol, thiol or secondary amine, eg.



X = Halogen, OH, COOH, SH, NHR

Similarly, the ease of linker synthesis also means that any functionalised resin may be used to immobilise the linker, eg. MBHA resin, amino or carboxy tentagel resins, 4-sulfamylbenzoyl AM resin etc.

C-Acylation of dimedone with, for example, a mono-protected di-carboxylic acid is readily achieved via a carbodiimide activated, DMAP catalysed condensation in dry DCM. Removal of the ester protection and coupling of the first amino sugar residue generates a sugar-linker conjugate which can be coupled readily to an amino-functionalised resin support via a carbodiimide-mediated

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condensation. This reaction can be monitored using conventional amine tests such as TNBS or ninhydrin, to ensure quantitative acylation. Alternatively, the linker can be synthesised directly on the resin, followed by  
5 introduction of the first sugar residue on to the linker-resin conjugate. Both methods are illustrated in Figure 3.

If an ether-type linkage between the primary sugar residue and the resin is required, then modification of the linker with 4-aminobenzylalcohol to generate the  
10 ODmab-type entity allows this method of chemical ligation, as illustrated in Figure 4.

Following selective deprotection of one hydroxyl group, the second sugar residue is coupled using any of the sugar donors referred to above, as illustrated in Figure 8.  
15 A portion of the resin is readily cleaved using either ammonia, hydrazine or a primary amine, as shown in Figure 5, and the cleavage mixture is analysed by TLC to monitor the reaction progress. Completion of the reaction is indicated by the disappearance of the monosaccharide. The  
20 sequential deprotection and coupling of the following sugar residues is continued until the desired oligosaccharide is complete, as illustrated in Figure 1. The protecting groups are then removed, and the oligosaccharide is cleaved from the resin support using either ammonia, hydrazine, or  
25 a primary amine, in a suitable solvent.

The resin-linker system of the invention is ideal for the synthesis of combinatorial oligosaccharide libraries, as shown in Figure 2, and for the immobilisation of mono- or oligosaccharides, as shown in  
30 Figure 7.

The invention will now be described in detail by way of reference only to the following non-limiting examples.

Examples 1-5      Synthesis of a Specially Protected  
Thioglycoside-Type Sugar Donor (Figure 9)

1      *Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-*  
*galactopyranoside*

5                    A mixture of galactose pentaacetate (38.00 g,  
97.43 mmol), (ethylthio)trimethylsilane (19.60 g,  
146.15 mmol) and trimethylsilyl trifluoromethanesulfonate  
(23.60 g, 106.20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (150 ml) was stirred  
overnight at room temperature. The reaction mixture was  
10 diluted with CH<sub>2</sub>Cl<sub>2</sub> (150 ml) and washed with 1M Na<sub>2</sub>CO<sub>3</sub>  
solution (300 ml), water (300 ml), dried over MgSO<sub>4</sub> and  
concentrated. The residue was crystallised from hexane/di-  
isopropyl ether 1:1 (v/v) to give ethyl 2,3,4,6-tetra-O-  
acetyl-1-thio-β-D-galactopyranoside (34.00 g, 89%).

15

R<sub>f</sub> 0.43 (hexane/EtOAc 1:1); FAB MS C<sub>16</sub>H<sub>24</sub>O<sub>9</sub>S (392.3) m/z (%)  
415 [M+Na]<sup>+</sup> (100), 393 [M+H]<sup>+</sup> (20), 331 (56).

2      *Ethyl 4,6-O-benzylidene-1-thio-β-D-galacto-pyranoside*

20                    A mixture of ethyl 2,3,4,6-tetra-O-acetyl-1-thio-  
β-D-galactopyranoside (10 g, 25.51 mmol) and sodium  
methoxide (200 mg, 3.7 mmol) was stirred in abs. MeOH  
(100 ml) at room temperature for 2 hours. The reaction  
mixture was neutralised with Amberlite IRA 120 (H<sup>+</sup>) ion  
25 exchange resin and evaporated. The residue was taken up in  
the (1:?) mixture of benzaldehyde/formic acid (21.2 ml) and  
stirred at room temperature for 90 minutes. The reaction  
mixture was diluted with ether (200 ml) and kept at -15°C  
for 2 hours. The precipitate formed was collected and  
30 purified by chromatography using CHCl<sub>3</sub>/ethanol 10:3 (v/v)  
to give ethyl 4,6-O-benzylidene-1-thio-β-D-galacto-  
pyranoside (8.1 g, 64.5%).

R<sub>f</sub> 0.64 (CHCl<sub>3</sub>/ethanol 10:3).

35

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3    *Ethyl 2,3-di-O-benzyl-4,6-O-benzylidene- $\beta$ -D-galactopyranoside*

          Ethyl 4,6-O-benzylidene-1-thio- $\beta$ -D-galactopyranoside (6.90 g, 22.11 mmol) in 60 ml DMF was added  
5    dropwise at 0°C to a suspension of sodium hydride 60% (2.65 g, 66.34 mmol) in 60 ml DMF. The mixture was stirred at room temperature for 1 hour, then benzyl bromide (11.34 g, 66.34 mmol) was added dropwise at 0°C. The mixture was stirred at room temperature overnight. The  
10    mixture was evaporated, and xylene (2x50 ml) was distilled from the residue. The residue was taken up in ether (300 ml) and washed with 2x100 ml water. The organic layer was dried over MgSO<sub>4</sub>, evaporated and crystallized from MeOH giving ethyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio- $\beta$ -D-galactopyranoside (8.90 g, 82%).  
15

R<sub>f</sub> 0.51 hexane/EtOAc 1:1 v/v); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.55-7.25 (m, 15H, 15 Ar-H), 5.47 (s, 1H, CHAr), 4.88-4.75 (4d, 4H, 2 CH<sub>2</sub>Ar), 4.44 (d, 1H, H-1, J<sub>1,2</sub>=10.89 Hz), 4.30 (dd, 1H, H-6'), 4.16 (d, 1H, H-4), (3.97 (dd, 1H, H-3), 3.88 (t, 1H, H-2), 3.60 (dd, 1H, H-6), 3.35 (d, 1H, H-5), 2.90-2.40 (m, 2H, CH<sub>2</sub>S), 1.33 (t, 3H, Me); FAB MS C<sub>28</sub>H<sub>32</sub>O<sub>5</sub>S (492.40) m/z (%) 515 [M+Na]<sup>+</sup> (100), 493 [M+H]<sup>+</sup> (41), 431 (53).  
20

25    4    *Ethyl 2,3,6-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside*

          A mixture of crude ethyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio- $\beta$ -D-galactopyranoside (5.4 g, 10.97 mmol), sodium cyanoborohydride (6.89 g, 109.7 mmol) and a few grains of methyl orange indicator was stirred in  
30    THF (60 ml) at 0°C. THF saturated with HCl was added very slowly until a permanent pink colour was obtained. The reaction mixture was stirred at room temperature for 20 min, then neutralised with dry NH<sub>3</sub> and evaporated. The residue was taken up in CHCl<sub>3</sub> (100 ml), washed with  
35    saturated NaHCO<sub>3</sub> solution (50 ml), dried over MgSO<sub>4</sub> and evaporated. The residue was dissolved in MeOH (50 ml), reflux for 10 min and evaporated. The crude product was

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purified by chromatography using 1,2-dichloroethane/ethyl acetate 10:0.5 as the mobile phase to give methyl 2,3,6-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside (4.14 g, 75%).

- 5  $R_f$  0.43 (1,2-dichloroethane/EtOAc 10:0.5 v/v);  $^1H$  NMR (CDCl<sub>3</sub>)  $\delta$  7.40-7.26 (m, 15H, 15 Ar-H), 4.88, 4.76, 4.73, 4.71 (4d, 4H, 2 CH<sub>2</sub>Ar), 4.57 (s, 2H, CH<sub>2</sub>Ar), 4.42 (d, 1H, H-1,  $J_{1,2}$ =9.64 Hz), 4.10 (m, 1H, H-4), (3.76 (dd, 1H, H-3), 3.67 (t, 1H, H-2), 3.55 (m, 2H, H-6), 2.75 (m, 2H, CH<sub>2</sub>S), 10 2.50 (bs, 1H, OH), 1.31 (t, 3H, CH<sub>3</sub>); FAB MS C<sub>29</sub>H<sub>34</sub>O<sub>5</sub>S (494.61) m/z (%) 627 [M+Cs]<sup>+</sup> (70), 517 [M+Na]<sup>+</sup> (30), 495 [M+H]<sup>+</sup> (12).

5 *Ethyl 2,3,6-tri-O-benzyl-4-bromoacetyl-1-thio- $\beta$ -D-*  
15 *galactopyranoside*

- A mixture of ethyl 2,3,6-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside (4.14 g, 8.38 mmol), sym. collidine (3.65 g, 30.16 mmol), and 4-dimethylaminopyridine in dry CH<sub>2</sub>Cl<sub>2</sub> (60 ml) was stirred at 0°C and bromoacetyl bromide 20 (2.53, 2.57 mmol) in CH<sub>2</sub>Cl<sub>2</sub> added dropwise in 15 minutes. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (100 ml) and washed with 5% HCl solution (3x30 ml) and saturated NaHCO<sub>3</sub> solution (30 ml). The solution was dried over MgSO<sub>4</sub> and evaporated. The residue was purified by chromatography 25 using hexane/EtOAc 2:1 as the mobile phase to give ethyl 2,3,6-tri-O-benzyl-4-bromoacetyl-1-thio- $\beta$ -D-galactopyranoside (4.84 g, 94%)

- $^1H$  NMR (CDCl<sub>3</sub>)  $\delta$  7.40-7.25 (m, 15H, 15 Ar-H), 4.80-4.50 (m, 30 6H, 3 CH<sub>2</sub>Ar), 4.45 (d, 1H, H-1,  $J_{1,2}$ =9.53 Hz), 2.73 (m, 2H, CH<sub>2</sub>S), 1.30 (t, 3H, CH<sub>3</sub>); FAB MS C<sub>31</sub>H<sub>35</sub>BrO<sub>6</sub>S (615.56) m/z (%) 638 [M+Na]<sup>+</sup> (15), 616 [M+H]<sup>+</sup> (32), 509 (80), 463 (21), 419 (18).

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Examples 6-10    Synthesis of a Partially-Protected Glycosyl  
Amine (Figure 9)

- 6     2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl azide  
1,2,3,4,6-penta-O-acetyl-galactopyranose (1.17 g,  
5   3 mmol) was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (15 ml), then  
trimethylsilyl azide (416 mg, 3.6 mmol) and  $\text{SnCl}_4$  (0.18 ml)  
were added under nitrogen. The mixture was stirred at room  
temperature for 24 hours. The reaction mixture was  
subsequently diluted with  $\text{CH}_2\text{Cl}_2$  (40 ml), dried over  $\text{MgSO}_4$ ,  
10 and evaporated. The residue was purified by chromatography  
using hexane/EtOAc 8:7 v/v as the mobile phase to give  
2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl azide (1.05 g,  
94%).
- 15    $R_f$  0.74 (hexane/EtOAc 8:7 v/v);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.41 (d,  
1H, H-4), 5.17 (t, 1H, H-2), 5.04 (dd, 1H, H-3), 4.60  
(d, 1H, H-1,  $J_{1,2}=10.09$  Hz), 4.19 (m, 2H, H-6), 4.00 (m, 1H,  
H-5), 2.15-1.98 (4s, 12H, 4 OAc); FAB MS  $\text{C}_{14}\text{H}_{19}\text{N}_3\text{O}_9$  (373.32)  
m/z (%) 396  $[\text{M}+\text{Na}]^+$  (100), 374  $[\text{M}+\text{H}]^+$  (35), 331 (23).
- 20   7   4,6-O-benzylidene- $\beta$ -D-galactopyranosyl azide  
A mixture of 2,3,4,6-tetra-O-acetyl- $\beta$ -D-galacto-  
pyranosyl azide (19.35 g, 51.79 mmol) and sodium methoxide  
(200 mg, 3.7 mmol) was stirred in abs. MeOH (100 ml) at  
25 room temperature for 2 hours. The reaction mixture was  
neutralised with Amberlite IRA 120 (H+) ion exchange resin  
and evaporated. The residue was taken up in the mixture of  
benzaldehyde/formic acid (1:1) (52 ml) and stirred at room  
temperature for 90 minutes. The reaction mixture was  
30 evaporated and the residue was taken up in ether (60 ml)  
and kept at  $-15^\circ\text{C}$  for 2 hours. The precipitate formed was  
collected by filtration and dried at room temperature  
affording 4,6-O-benzylidene- $\beta$ -D-galactopyranosyl azide  
(11.8 g 78%).
- 35    $R_f$  0.64 ( $\text{CHCl}_3$ /ethanol 10:1.5).

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8    2,3-di-O-benzyl-4,6-O-benzylidene- $\beta$ -D-galactopyranosyl  
azide

          4,6-O-benzylidene- $\beta$ -D-galactopyranosyl azide  
(11.8 g, 40.27 mmol) in 60 ml DMF was added dropwise at 0°C  
5    to a suspension of sodium hydride 60% (6.21 g, 155.38 mmol)  
in 60 ml DMF. The mixture was stirred at room temperature  
for 1 hour, then benzyl bromide (26.57 g, 155.38 mmol) was  
added dropwise at 0°C. The mixture was stirred at room  
temperature overnight. The mixture was evaporated, and  
10    xylene (2x50 ml) was distilled from the residue. The  
residue was taken up in ether (500 ml) and washed with  
2x100 ml water. The organic layer was dried over MgSO<sub>4</sub> and  
evaporated, giving methyl 2,3-di-O-benzyl-4,6-O-  
benzylidene- $\beta$ -D-galactopyranosyl azide as a crude residue  
15    (19.4 g).

9    2,3,6-tri-O-benzyl- $\beta$ -D-galactopyranosyl azide

          A mixture of crude 2,3-di-O-benzyl-4,6-O-  
benzylidene- $\beta$ -D-galactopyranosyl azide (9.00 g,  
20    19.02 mmol), sodium cyanoborohydride (12.00 g, 190.2 mmol)  
and a few grains of methyl orange indicator was stirred in  
THF (80 ml) at 0°C. THF saturated with HCl was added very  
slowly until a permanent pink colour was obtained. The  
reaction mixture was stirred at room temperature for  
25    20 min, then neutralised with dry NH<sub>3</sub> and evaporated. The  
residue was taken up in CHCl<sub>3</sub> (100 ml), washed with  
saturated NaHCO<sub>3</sub> solution (50 ml), dried over MgSO<sub>4</sub> and  
evaporated. The residue was dissolved in MeOH (50 ml) and  
kept under reflux for 10 min and evaporated. The crude  
30    product was purified by chromatography using 1,2-dichloro-  
ethane/EtOAc 10:0.4 as the mobile phase to give 2,3,6-tri-  
O-benzyl- $\beta$ -D-galactopyranosyl azide (6.50 g, 72%).

R<sub>f</sub> 0.42 (1,2-dichloroethane/EtOAc 10:0.4 v/v); <sup>1</sup>H NMR  
35    (CDCl<sub>3</sub>)  $\delta$  7.40 (m, 15H, 15 Ar-H), 4.90-4.55 (m, 6H,  
3 CH<sub>2</sub>Ar), 4.06 (m, 1H, H-4), (3.82-3.70 (m, 3H, H-3, H-2,  
H-5), 3.65 (dd, 1H, H-6'), 3.60 (d, 1H, H-1, J<sub>1,2</sub> = 8.64 Hz),

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3.51 (dd, 1-H, H-6); FAB MS  $C_{27}H_{29}N_3O_5$  (475.40) m/z (%) 608 [M+Cs]<sup>+</sup> (10), 498 [M+Na]<sup>+</sup> (65), 476 [M+H]<sup>+</sup> (25), 433 (75), 341 (20).

5 10 2,3,6-tri-O-benzyl- $\beta$ -D-galactopyranosyl amine

A mixture of 2,3,6-tri-O-benzyl- $\beta$ -D-galactopyranosyl azide (3.00 g, 6.31 mmol), propane-1,3-dithiol (3.40 g, 31.50 mmol), and triethylamine (3.50 g, 31.5 mmol) in MeOH (31 ml) was stirred under nitrogen at room temperature for 10 hours. The reaction mixture was evaporated and purified by chromatography using  $CHCl_3$ /EtOH 10:0.3 v/v to give 2,3,6-tri-O-benzyl- $\beta$ -D-galactopyranosyl amine (2.66 g, 94%);

15  $R_f$  0.38 ( $CHCl_3$ /EtOH 10:0.3 v/v); FAB MS  $C_{27}H_{31}NO_5$  (449.33) m/z (%) 472 [M+Na]<sup>+</sup> (75), 450 [M+H]<sup>+</sup> (100).

Example 11                      Synthesis of a Glycosyl Amine - Ddh-Benzyl Ester Conjugate in Solution (Figure 3)

20 11 N-(Benzyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl amine

A mixture of benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (932 mg, 2.60 mmol), 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl amine in  $CH_2Cl_2$  (2.0 ml) was stirred at room temperature for 2 days. The reaction mixture was evaporated and purified by chromatography using hexane/EtOAc 1:1 as the mobile phase to give N-(Benzyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl amine (1.70 g, 95%);

$R_f$  0.32 (hexane/EtOAc 1:1 v/v); <sup>1</sup>H NMR ( $CDCl_3$ )  $\delta$  7.37-7.26 (m, 5H, 5 Ar-H), 5.40-5.00 (m, 7H, 7 sugar protons), 3.10, 2.85 (2t, 4H, 2  $CH_2$ ), 2.38 (2s, 4H, Dde 2  $CH_2$ ), 2.06-1.98 (4s, 12H, 4 OAc), 1.80 (m, 4H, 2  $CH_2$ ), 1.02, 1.00 (2s, 6H,

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Dde 2CH<sub>3</sub>); FAB MS C<sub>35</sub>H<sub>45</sub>NO<sub>13</sub> (687.23) m/z (%) 710 [M+Na]<sup>+</sup> (35), 688 [M+H]<sup>+</sup> (100), 356 (60).

Example 12                      Synthesis of a Fully Protected Glycosyl  
5                                      Amine - Ddh Conjugate Deprotecting a "Fully  
   Protected Amine - DdH Ester Conjugate" in  
   Solution (Figure 3)

12        N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic  
                 acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl  
10        amine

                 N-(Benzyl 6-(4,4-dimethyl-2,6-dioxocyclo-  
                 hexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-  
                 glucopyranosyl amine (1.27 g, 1.84 mmol) was hydrogenated  
                 over Pd/C (10%) (200 mg) in MeOH (20 ml) at room  
15        temperature for 10 hours. The catalyst was filtered off,  
                 and the filtrate was evaporated and then chromatographed  
                 using CHCl<sub>3</sub>/MeOH 10:0.5 v/v as the mobile phase to give  
                 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-  
                 6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine  
20        1.10 g, 98%);

                 R<sub>f</sub> 0.38 (CHCl<sub>3</sub>/MeOH 10:0.5 v/v); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 5.40-5.00  
                 (m, 7H, 7 sugar protons), 3.15, 2.86 (2t, 4H, 2 CH<sub>2</sub>), 2.45  
                 (2s, 4H, Dde 2 CH<sub>2</sub>), 2.10-1.98 (4s, 12H, 4 OAc), 1.80-1.65  
25        (m, 4H, 2 CH<sub>2</sub>), 1.02, 1.00 (2s, 6H, Dde 2CH<sub>3</sub>); FAB MS  
                 C<sub>28</sub>H<sub>39</sub>NO<sub>13</sub> (597.33) m/z (%) 620 [M+Na]<sup>+</sup> (55), 598 [M+H]<sup>+</sup>  
                 (100).

Example 13                      Synthesis of a Glycosyl Amine - Ddh-Methyl  
30                                      Ester Conjugate in Solution (Figure 3)

13        N-(Methyl 6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-  
                 hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-  
                 glucopyranosyl amine

                 Reaction 11 was repeated with the difference that  
35        methyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-  
                 hexanoate was used instead of benzyl 6-hydroxy-6-(4,4-  
                 dimethyl-2,6-dioxocyclohexylidene)-hexanoate. Yield: 92%;

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$R_f$  0.28 (hexane/EtOAc 1:1 v/v); FAB MS  $C_{29}H_{41}NO_{13}$  (611.45)  
m/z (%) 624  $[M+Na]^+$  (100), 612  $[M+H]^+$  (34).

5    Example 14                    Synthesis of a Glycosyl Amine - Ddh-t-Butyl  
   Ester Conjugate in Solution (Figure 3)

14    *N*-(*t*-Butyl 6-(4,4-dimethyl-2,6-dioxocyclo-  
   hexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-*O*-acetyl- $\beta$ -  
   *D*-glucopyranosyl amine

10                    Reaction 11 was repeated with the difference that  
                         *t*-butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-  
                         hexylidene)-hexanoate was used instead of benzyl 6-hydroxy-  
                         6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate. Yield:  
                         96%;

15                     $R_f$  0.35 (hexane/EtOAc 1:1 v/v); FAB MS  $C_{32}H_{47}NO_{13}$  (653.37)  
                         m/z (%) 676  $[M+Na]^+$  (80), 677  $[M+H]^+$  (100).

20    Example 15                    Synthesis of Ddh-OH Benzyl Ester in  
   Solution (Figure 3)

15    Benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxo-  
   cyclohexylidene)-hexanoate

                         To a stirred solution of mono-benzyl adipate  
                         (2.36g, 10 mmol) in dry  $CH_2Cl_2$  (50 ml) was added 5,5-  
25    dimethyl-1,3-cyclohexanedione (1.4 g, 10 mmol), *N,N'*-  
                         dicyclohexylcarbodiimide (2.1 g, 10.1 mmol) and  
                         4-dimethylaminopyridine (1.22 g, 10 mmol). The resulting  
                         solution was allowed to stir at room temperature for 18 h.  
                         The solution was cooled and filtered to remove the  
30    precipitated *N,N'*-dicyclohexylurea. The filtrate was  
                         evaporated and the residue redissolved in EtOAc (50 ml) and  
                         washed with 1 M  $KHSO_4$ . The organic extract was washed with  
                         brine (92x10 ml), dried ( $MgSO_4$ ) and evaporated to yield a  
                         white/yellow amorphous powder. Flash silica chromatography  
35    (EtOAc/hexane 1:2 v/v) afforded benzyl 6-hydroxy-6-(4,4-  
                         dimethyl-2,6-dioxocyclohexylidene)-hexanoate (3.00 g, 84%)  
                         as a white crystalline solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 18.10 (s, 1H, OH), 7.30 (s, 5H, 5Ar-H), 5.06 (s, 2H, CH<sub>2</sub>Ar), 3.00 (t, 2H, CH<sub>2</sub>), 2.47 (s, 2H, Dde CH<sub>2</sub>), 2.35 (t, 2H, CH<sub>2</sub>CO<sub>2</sub>), 2.29 (s, 2H, Dde CH<sub>2</sub>), 1.65 (m, 4H, 2 CH<sub>2</sub>), 1.01 (s, 6H, 2 CH<sub>3</sub>); FAB MS C<sub>21</sub>H<sub>26</sub>O<sub>5</sub> (358.18) m/z (%) 359 [M+H]<sup>+</sup> (100), 267 (40); HRMS (FAB) Found: m/z 359.1858 Calcd for C<sub>21</sub>H<sub>27</sub>O<sub>5</sub>: (M+H), 359.1850.

10 Example 16      Synthesis of Ddh-OH by Deprotection of a Ddh-OH Ester (Figure 3)

16      6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid

Benzy 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoate (1.50 g, 4.19 mmol) was hydrogenated over Pd/C (10 %) (150 mg) in MeOH (20 ml) at room temperature for 10 hours. The catalyst was filtered off, and the filtrate was evaporated, yielding 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid (1.10 g, 98%);

R<sub>f</sub> 0.35 (hexane/EtOAc 2:1 v/v); FAB MS C<sub>14</sub>H<sub>20</sub>O<sub>5</sub> (268.12) m/z (%) 313 [M+2Na]<sup>+</sup> (34), 291 [M+Na]<sup>+</sup> (100), 269 [M+H]<sup>+</sup> (16).

25 Example 17      Synthesis of a Ddh-OH Methyl Ester in Solution (Figure 3)

17      Methyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-hexanoate

Reaction 15 was repeated, with the difference that mono-methyl adipate was used instead of mono-benzyl adipate, and afforded methyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (2.39 g, 85%).

R<sub>f</sub> 0.32 (EtOAc/hexane 1:2 v/v) FAB MS C<sub>15</sub>H<sub>22</sub>O<sub>5</sub> (282.22) m/z (%) 305 [M+H]<sup>+</sup> (100), 283 [M+H]<sup>+</sup> (66).

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Example 18                    Synthesis of Ddh-OH t-Butyl Ester in  
Solution (Figure 3)

18     *t*-Butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-  
hexylidene)-hexanoate

5                    Reaction 15 was repeated, with the difference  
that mono-*t*-butyl adipate was used instead of mono-benzyl  
adipate, and afforded *t*-butyl 6-hydroxy-6-(4,4-dimethyl-  
2,6-dioxocyclohexylidene)-hexanoate (2.62 g, 81%).

10      $R_f$  0.36 (EtOAc/hexane 1:2 v/v) FAB MS  $C_{18}H_{28}O_7$  (324.41)  $m/z$   
(%) 347  $[M+H]^+$  (100), 325  $[M+H]^+$  (43), 267 (80).

Example 19                    Synthesis of Ddh-OH by Deprotection of a  
Ddh-OH t-Butyl Ester (see 16, Figure 3)

15     19     6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-  
hexanoic acid

*t*-Butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-  
hexylidene)-hexanoate ( 100 mg, 0.30 mmol) was dissolved in  
CH<sub>2</sub>Cl<sub>2</sub>/TFA 1:1 mixture (2 ml) and stirred at room  
20     temperature for 1 h. The reaction mixture was evaporated  
giving 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-  
hexanoic acid (0.81 g, 98%)

Example 20                    Synthesis of Ddh-OH from Cyclic Anhydrides  
(see 16, Figure 3)

25                    20     6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-  
hexanoic acid

                  A mixture of glutaric anhydride (2.28 g,  
20 mmol), dimedone (2.8 g, 20 mmol), 4-dimethylamino-  
30     pyridine (3.99 g, 30 mmol) in abs. pyridine (50 ml) was  
stirred at room temperature for 24 h. The reaction mixture  
was evaporated and the residue was taken up in CHCl<sub>3</sub>,  
(100 ml), washed 5% HCl solution (3x25 ml), saturated  
NaHCO<sub>3</sub> solution, dried over MgSO<sub>4</sub> and evaporated. The  
35     residue was purified by chromatography using ether/acetic  
acid (10 ml:1 drop) as the mobile phase to give 6-hydroxy-

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6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid  
(2.28 g, 45%).

Example 21                      Synthesis of a Fully Protected Glycosyl  
5                                      Amine - Ddh Conjugate Using Ddh-OH in  
   Solution (See 12, Figure 3)

21        *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic  
   acid-6-yl) 2,3,4,6-tetra-*O*-acetyl- $\beta$ -*D*-glucopyranosyl  
   amine

10                      A mixture of 6-hydroxy-6-(4,4-dimethyl-2,6-  
   dioxocyclohexylidene)-hexanoic acid (400 mg, 1.49 mmol),  
   2,3,4,6-tetra-*O*-acetyl- $\beta$ -*D*-glucopyranosyl amine (259 mg,  
   0.74 mmol) in abs. EtOH was stirred under reflux for 2 h.  
   The reaction mixture was evaporated and chromatographed  
15                      using CHCl<sub>3</sub>/MeOH 10:0.5 v/v to give *N*-(6-(4,4-dimethyl-2,6-  
   dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-*O*-  
   acetyl- $\beta$ -*D*-glucopyranosyl amine (410 mg, 92%).

Example 22                      Synthesis of a Partially Protected Glycosyl  
20                                      Amine - Ddh Conjugate Using Ddh-OH in  
   Solution (Figure 3)

22        *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic  
   acid-6-yl) 2,3,6-tri-*O*-benzyl- $\beta$ -*D*-galactopyranosyl  
   amine

25                      Reaction 21 was repeated with the difference that  
   2,3,6-tri-*O*-benzyl- $\beta$ -*D*-galactopyranosyl amine was used  
   instead of 2,3,4,6-tetra-*O*-acetyl- $\beta$ -*D*-glucopyranosyl amine,  
   and afforded *N*-(6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-  
   hexanoic acid-6-yl) 2,3,6-tri-*O*-benzyl- $\beta$ -*D*-galactopyranosyl  
30                      amine (299 mg, 90%).

R<sub>f</sub> 0.34 (CHCl<sub>3</sub>/MeOH 10:0.1 v/v) FAB MS C<sub>37</sub>H<sub>43</sub>NO<sub>7</sub> (613.41) m/z  
(%) 649 [M+2Na]<sup>+</sup> (34), 626 [M+Na]<sup>+</sup> (100), 614 [M+H]<sup>+</sup> (65).

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Example 23      Synthesis of Ddh-Aminobenzyl Linker in  
Solution (Figure 4)

23      *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic  
acid-6-yl) 4-amino-benzylalcohol

5              Reaction 21 was repeated with the difference that  
4-aminobenzyl alcohol was used instead of 2,3,4,6-tetra-O-  
acetyl- $\beta$ -D-glucopyranosyl amine, and afforded *N*-(6-(4,4-  
dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-  
aminobenzyl alcohol (259 mg, 94%).

10

$R_f$  0.40 (EtOAc/hexane/acetic acid 2:1:0.1 v/v/v); FAB MS  
 $C_{21}H_{27}NO_6$  (373.43)  $m/z$  (%) 418  $[M+2Na]^+$  (24), 396  $[M+Na]^+$   
(100), 374  $[M+H]^+$  (35).

15      Example 24      Synthesis of Ddh-Aminobenzyl t-Butyl Ester  
Linker in Solution (Figure 4)

24      *N*-(*t*-Butyl 6-(4,4-dimethyl-2,6-dioxocyclo-  
hexylidene)-hexanoate-6-yl) 4-aminobenzyl alcohol

20              A mixture of *t*-butyl 6-hydroxy-6-(4,4-dimethyl-  
2,6-dioxocyclohexylidene)-hexanoate (400 mg, 1.23 mmol) and  
4-aminobenzyl alcohol (605 mg, 4.92 mmol) in abs. EtOH was  
stirred under reflux for 2 h. The reaction mixture was  
evaporated and purified by chromatography using  $CHCl_3/MeOH$   
9:1 as the mobile phase to give *N*-(*t*-Butyl 6-(4,4-dimethyl-  
25      2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl  
alcohol (395 mg, 75%)

$R_f$  0.52 ( $CHCl_3/MeOH$  9:1 v/v) FAB MS  $C_{25}H_{35}NO_6$  (429.53)  $m/z$   
(%) 452  $[M+Na]^+$  (100), 430  $[M+H]^+$  (32), 372 (64).

30

Example 25                    Synthesis of Ddh-Aminobenzyl t-Butyl Ester  
Trichloroacetimidate Activated Linker in  
Solution (Figure 4)

25     *N*-(*t*-Butyl 6-(4,4-dimethyl-2,6-dioxocyclo-  
5        hexylidene)-hexanoate-6-yl) 4-aminobenzyl  
         trichloroacetimidate

         A mixture of *N*-(*t*-butyl 6-(4,4-dimethyl-2,6-  
dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl alcohol  
(500 mg, 1.16 mmol) and trichloroacetonitrile (503 mg,  
10     3.49 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 ml) was stirred at 0°C and 1,8-  
diazabicyclo(5.4.0)undec-7-ene (5 mg, 0.03 mmol) added.  
The reaction mixture was stirred at 0°C for 90 minutes, at  
room temperature for 2 h, then evaporated. The residue was  
15     purified by chromatography using EtOAc/hexane 1:1 as the  
mobile phase to give *N*-(*t*-butyl 6-(4,4-dimethyl-2,6-  
dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl  
trichloroacetimidate (580 mg, 87%);

*R<sub>f</sub>* 0.41 (EtOAc/hexane 1:1 v/v); FAB MS C<sub>27</sub>H<sub>35</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>5</sub> (573.94)  
20     *m/z* (%) 595 [M+Na]<sup>+</sup> (100), 753 [M+H]<sup>+</sup> (40), 515 (39), 430  
(54).

Example 26                    Synthesis of a Fully Protected Sugar  
25                    (Sugar-Linker Bond is not at the Glycosidic  
                         Position) - Ddh-Aminobenzyl t-Butyl Ester  
                         Conjugate Via Trichloroacetimidate  
                         Activation (Figure 4)

26     Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-  
         [N-(*t*-butyl 6-(4,4-dimethyl-2,6-dioxocyclo-  
30        hexylidene)-hexanoate-6-yl) 4-aminobenzyl]-α-D-  
         glucopyranoside

         N-(*t*-Butyl 6-(4,4-dimethyl-2,6-dioxocyclo-  
hexylidene)-hexanoate-6-yl) 4-aminobenzyl trichloro-  
acetimidate (400 mg, 0.70 mmol) was added at 20°C under  
35     nitrogen to a solution of Benzyl 2-acetamido-3-O-acetyl-6-  
O-benzyl-2-deoxy-α-D-glucopyranoside (155 mg, 0.35 mmol) in  
CH<sub>2</sub>Cl<sub>2</sub> (6 ml). Trifluoromethanesulphonic acid in ether

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(0.1 M, 0.06 ml) was added and the mixture was stirred for 30 min at 20°C. The reaction was stopped with 5% NaHCO<sub>3</sub> solution (0.25 ml). After filtration of the mixture and evaporation of the filtrate, the crude residue was purified by chromatography using EtOAc/hexane 2:1 v/v as the mobile phase to give Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-[N-(t-butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl]- $\alpha$ -D-glucopyranoside (210 mg, 71%).

R<sub>f</sub> 0.37 (EtOAc/hexane 2:1 v/v); FAB MS C<sub>49</sub>H<sub>62</sub>N<sub>2</sub>O<sub>11</sub> (855.01) m/z (%) 877 [M+Na]<sup>+</sup> (100), 855 [M+H]<sup>+</sup> (35), 797 (73).

Example 27                      Synthesis of a Fully Protected Glycoside  
(Sugar-Linker Bond at the Glycosidic  
Position) - Ddh-Aminobenzyl Linker - Resin  
Via Trichloroacetimidate Activation  
(Figure 4)

[N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-aminobenzyl] 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranoside MBHA resin conjugate

N-(t-Butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl trichloroacetimidate (400 mg, 0.70 mmol) was added at 20°C under nitrogen to a solution of 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranose (121 mg, 0.35 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 ml). Trifluoromethanesulphonic acid in ether (0.1 M, 0.06 ml) was added and the mixture was stirred for 30 min at 20°C. The reaction was stopped with 5% NaHCO<sub>3</sub> solution (0.25 ml). After filtration of the mixture, the filtrate was evaporated. The unpurified residue was taken up in CH<sub>2</sub>Cl<sub>2</sub>/TFA mixture (1:1) (5 ml), stirred at room temperature for 1 h and evaporated. The resulting acid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 ml), N,N'-diisopropylcarbodiimide (128 mg, 1 mmol) added, and the mixture was gently agitated with MBHA resin (100 mg) (swelled in DMF for 20 min.) for 30 min.

### Example 28

Synthesis of a Fully Protected Glycoside  
(Sugar - Linker Bond is at the Glycoside  
Position) - Ddh-Aminobenzyl Benzyl Ester  
Conjugate Via DMTST Promoted Glycosylation  
(see 26, Figure 4)

28 [N-[Benzyl (6-(4,4-dimethyl-2,6-dioxocyclo-  
hexylidene)-hexanoate]-6-yl 4-aminobenzyl]-2,3,4,6-  
tetra-O-acetyl- $\beta$ -D-glucopyranoside

10 A mixture of N-[Benzyl (6-(4,4-dimethyl-2,6-  
dioxocyclohexylidene)-hexanoate]-6-yl 4-aminobenzyl alcohol  
(500 mg, 1.08 mmol), methyl 2,3,4,6-tetra-O-acetyl-1-thio-  
15  $\beta$ -D-glucopyranoside (400 mg, 1.08 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 ml)  
was stirred at room temperature and DMTST (835 mg,  
3.24 mmol) added. The solution was stirred at room  
temperature for 1 h and washed with saturated  $\text{NaHCO}_3$   
solution (3 ml), dried over  $\text{MgSO}_4$  and evaporated. The  
residue was purified by chromatography using hexane/EtOAc  
1:1 v/v as the mobile phase to give [N-[Benzyl (6-(4,4-  
20 dimethyl-2,6-dioxocyclohexylidene)-hexanoate]-6-yl 4-  
aminobenzyl]-2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranoside  
(610 mg, 75%).

25  $R_f$  0.47 (hexane/EtOAc 1:1 v/v); FAB MS  $C_{42}H_{51}NO_{14}$  (793.83)  
m/z (%) 816  $[M+Na]^+$  (100), 794  $[M+H]^+$  (25), 702 (66).

### Example 29

Synthesis of a Fully Protected Glycoside (Sugar-Linker Bond is at the Glycosidic Position) - Ddh-Aminobenzyl Linker - Resin Conjugate Via DIPCDI Activation (see 27, Figure 4)

29 [N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-  
hexanoic acid-6-yl) 4-aminobenzyl]-2,3,4,6-tetra-O-  
acetyl- $\beta$ -D-glucopyranoside MBHA resin conjugate  
35 [N-[Benzyl (6-(4,4-dimethyl-2,6-dioxocyclo-  
hexylidene)-hexanoate)-6-yl 4-aminobenzyl]-2,3,4,6-tetra-O-  
acetyl- $\beta$ -D-glucopyranoside (500 mg, 0.63 mmol) was

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hydrogenated over Pd/C (10%) (200 mg) in MeOH (20 ml) at room temperature for 10 hours. The catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 ml), N,N'-diisopropylcarbodiimide (128 mg, 1 mmol) added, and the mixture was gently agitated with MBHA resin (200 mg) (pre-swelled in DMF for 20 min.) for 30 min.

Example 30      Synthesis of a Partially Protected Glycosyl  
Amine - Ddh Conjugate Using Ddh-OH t-Butyl  
Ester in Solution (see 22, Figure 3)

30      *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine

15      A mixture of t-butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (400 mg, 1.23 mmol) and 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (276 mg, 0.61 mmol) in abs. EtOH (10 ml) was stirred under reflux for 2 h. The reaction mixture was evaporated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub>/TFA mixture (1:1) (10 ml) and stirred at room temperature for 1 h. The reaction mixture was evaporated and purified by chromatography using CHCl<sub>3</sub>/MeOH 10:0.1 v/v as the mobile phase to give *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (280 mg, 73%).

R<sub>f</sub> 0.34 (CHCl<sub>3</sub>/MeOH 10:0.1 v/v) FAB MS C<sub>37</sub>H<sub>43</sub>NO<sub>7</sub> (613.41) m/z (%) 649 [M+2Na]<sup>+</sup> (34), 626 [M+Na]<sup>+</sup> (100), 614 [M+H]<sup>+</sup> (65).

30

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Example 31      Synthesis of a Fully Protected Glycosyl  
Amine - Ddh - Resin Conjugate Where the  
Resin Coupling is the Final Step (Figure 3)

31      *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic  
5      acid-6-yl) 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl  
amine - MBHA conjugate

MBHA resin (Subst. ratio: 0.42 mmol/g) (200 mg)  
bearing a total amine functionality of 0.084 mmol was  
swollen in DMF for 20 min. The resin was then washed with  
10 fresh DMF and *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-  
hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl  
pyranosyl amine (200 mg, 4 equiv.) and *N,N'*-diisopropyl-  
carbodiimide (53  $\mu$ l, 4 equiv.) were added in DMF (5 ml) and  
the resin gently agitated for 30 min. The TNBS test was  
15 faintly positive so using the above conditions, a double  
coupling was performed, this time producing a negative TNBS  
test result. The resin was washed with DMF, methanol and  
finally ether. The resin was then allowed to dry in vacuum  
over KOH overnight.

20

Example 32      Synthesis of a Fully Protected Sugar (Sugar  
- Linker Bond is Not at the Glycosidic  
Position) - Ddh - Resin Conjugate Where the  
Resin Coupling is the Final Step (see 27,  
25 Figure 4)

32      Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-  
[*N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-  
hexanoic acid-6-yl) 4-aminobenzyl]- $\alpha$ -D-  
glucopyranoside - MBHA resin conjugate

30      Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-  
4-O-[*N*-(*t*-butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-  
hexanoate-6-yl) 4-aminobenzyl]- $\alpha$ -D-glucopyranoside (290 mg,  
0.33 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub>/TFA mixture (1:1) and  
stirred at room temperature for 1 h. The reaction mixture  
35 was evaporated, and procedure 31 was used to bind the  
compound to the MBHA resin.

Example 33      Synthesis of Ddh-Aminobenzyl Linker - Resin  
Conjugate With Selective Resin Coupling  
(Unprotected Hydroxyl Group is Present on  
the Linker) (Figure 10)

5    33    *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic  
acid-6-yl) 4-amino-benzylalcohol - MBHA resin  
conjugate

MBHA resin (100 mg) bearing a total amine  
functionality of 0.042 mmol was swelled in DMF for 20 min.  
10    The resin was then washed with fresh DMF and *N*-(6-(4,4-  
dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-  
aminobenzyl alcohol (63 mg, 4 equiv.) and 1-isobutyloxy-  
carbonyl-2-isobutyloxy-1,2-dihydroquinoline (EEDQ) (51 mg,  
4 equiv.) were added in DMF (5 ml) and the resin gently  
15    agitated for 24 h. The TNBS test was faintly positive so  
using the above conditions, a double coupling was  
performed, this time producing a negative TNBS test result.  
The resin was washed with DMF (5x10 ml).

20    Example 34      Synthesis of Ddh-Aminobenzyl  
Trichloroacetimidate Activated Linker -  
Resin Conjugate When the Activation Takes  
Place on the Resin (Figure 10)

34    *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-  
25    hexanoate-6-yl) 4-aminobenzyl trichloroacetimidate - MBHA  
resin conjugate

Resin from Example 33 was treated with  
trichloroacetonitrile (50 mg, 0.33 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 ml)  
was stirred at 0°C and 1,8-diazabicyclo(5.4.0)undec-7-ene  
30    (1 mg, 0.003 mmol) added. The reaction mixture was stirred  
at 0°C for 90 minutes, at room temperature for 2 h, then  
the resin was filtered off and washed with DMF (5x10 ml).

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Example 35      Synthesis of a Fully Protected Sugar (Sugar - Linker Bond is Not at the Glycosidic Position) - Ddh - Resin Conjugate When the Sugar Coupling is the Final Step (see 32, Figure 4)

35      *Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-[N-(6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid-6-yl) 4-aminobenzyl]- $\alpha$ -D-glucopyranoside - MBHA resin conjugate*

10              Resin from Example 34 was added at room temperature to a solution of Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy- $\alpha$ -D-glucopyranoside (75 mg, 0.16 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 ml). Trifluoromethanesulphonic acid in ether (0.1 M, 60  $\mu$ l) was added and the mixture was stirred for 15 30 min. The reaction was stopped with triethylamine (120  $\mu$ l) and washed with DMF (5x10 ml).

Example 36      First Step of the Solid Phase Synthesis of the Resin - Ddh- or DdH-Aminobenzyl - Linker (Figure 3)

20      36      *Adipic acid - MBHA resin conjugate*

            MBHA resin (1.0 g) bearing a total amine functionality of 0.42 mmol was swelled in DMF for 20 min. The resin was then treated with a mixture of adipic acid 25 (1.41 g, 10 mmol) and N,N'-diisopropylcarbodiimide in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) for 60 min. A second coupling was performed in DMF to get a negative ninhydrin test. The resin was washed with DMF (5x10 ml).

30      Example 37      Second Step of the Solid Phase Synthesis of the Resin - Ddh- or DdH-Aminobenzyl - Linker (Figure 3)

37      *6-Hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid - MBHA resin conjugate*

35              To the resin from Example 36 a mixture of 5,5-dimethyl-1,3-cyclohexanedione (280 mg, 2.0 mmol), N,N'-dicyclohexylcarbodiimide (283 mg, 2.00 mmol) and

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4-dimethylaminopyridine (244 mg, 2.00 mmol) was added in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) and stirred at room temperature for 18 h. The resin was washed with DMF (5x10 ml).

5    Example 38            Solid Phase Synthesis of a Fully Protected Glycosyl Amine - Ddh - Resin Conjugate (see 31, Figure 3)

38    *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl  
10    amine - MBHA resin conjugate

The resin from Example 37 was reacted with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine (712 mg, 2.00 mmol) in DMF (5 ml) at room temperature for 2 days. The resin was washed with DMF (5x10 ml).

15

Example 39            Solid Phase Synthesis of a Partially Protected Glycosyl Amine - Ddh - Resin Conjugate (Figure 3)

39    *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl  
20    amine - MBHA resin conjugate

The resin from Example 37 was reacted with 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (900 mg, 2.00 mmol) in abs. EtOH under reflux for 2 h. The resin was washed  
25    with DMF (5x10 ml).

Example 40            Solid Phase Synthesis of Ddh-Aminobenzyl Linker - Resin Conjugate (see 33, Figure 10)

30    40    *N*-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-amino-benzylalcohol - MBHA resin conjugate

A mixture of resin from Example 37 and 4-aminobenzyl alcohol (246 mg, 2.00 mmol) in abs. EtOH was  
35    stirred under reflux for 2 h, then washed with DMF (5x10 ml).

Example 41      Cleavage of a Fully Protected Glycosyl  
Amine - Ddh - Resin Conjugate Affording  
Fully Protected Glycosyl Amine (Figure 11)

41      *Cleavage of N-(6-(4,4-dimethyl-2,6-dioxocyclo-*  
5      *hexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-*  
*acetyl-β-D-glucopyranosyl amine - MBHA resin*  
*conjugate by NH<sub>3</sub> treatment.*

Resin from Example 38 (10 mg) was treated with  
saturated NH<sub>3</sub>/MeOH solution (0.2 ml) at room temperature  
10 for 5 min. The resin was filtered off, the filtrate was  
evaporated, giving 2,3,4,6-tetra-O-acetyl-β-D-  
glucopyranosyl amine in quantitative yield.

Example 42      Cleavage of a Fully Protected Glycosyl  
15      Amine - Ddh - Resin Conjugate Affording  
Fully Protected Reducing Sugar

42      *Cleavage of N-(6-(4,4-dimethyl-2,6-dioxocyclo-*  
*hexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-*  
*acetyl-β-D-glucopyranosyl amine - MBHA resin*  
20      *conjugate by NH<sub>3</sub> treatment, affording a reducing*  
*carbohydrate derivative (Figure 11).*

Resin from Example 38 (10 mg) was treated with  
saturated NH<sub>3</sub>/MeOH solution (0.2 ml) at room temperature  
for 5 min. The resin was filtered off, the filtrate was  
25 evaporated. The residue was dissolved in the mixture of  
acetone/water 10:1 v/v (0.2 ml), acidified with acetic acid  
(20 μl) and stirred at room temperature for 1 h. The  
solution was evaporated giving 2,3,4,6-tetra-O-acetyl-β-D-  
glucopyranose in quantitative yield.

Example 43      Carbohydrate Deprotection of the Fully  
30      Protected Sugar -Ddh Linker - Resin  
Conjugate (Figure 12)

43      *N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic*  
35      *acid-6-yl) β-D-glucopyranosyl amine - MBHA resin conjugate*

The resin from Example 38 was gently agitated  
with sodium methoxide (200 mg, 3.70 mmol) in abs. MeOH

(5 ml) at room temperature for 1 h. The resin was washed with abs. MeOH (5x10 ml), DMF (5x10 ml), ether (5x10 ml) and dried under high vacuum for 1 h, giving the resin-bonded unprotected  $\beta$ -D-glucopyranosyl amine. A sample of resin  
5 (5 mg) was cleaved by  $\text{NH}_3/\text{MeOH}$  (Example 41), and the resulting product was analyzed by TLC and mass spectrometry, proving the quantitative deprotection.

10 Example 44      Synthesis of a Library of Di-, Tri- and Tetrasaccharides on a Solid Support  
(Figure 12)

44 A mixture of mono-, di- and tri-O-(2,3,4-tri-O-benzyl  $\alpha,\beta$ -L-fucopyranosyl) (1 $\rightarrow$ 2), (1 $\rightarrow$ 3), (1 $\rightarrow$ 4), (1 $\rightarrow$ 6)-[N-(6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid-6-yl)]  $\beta$ -D-glucopyranosyl amine - MBHA resin  
15 conjugate

A mixture of resin from Example 43 and ethyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside (950 mg, 2 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (10 ml) was treated with dimethyl-(methylthio)-sulphonium trifluoromethanesulphonate (DMTST)  
20 (1.50 g, 5.81 mmol) at room temperature for 1 h. The resin was washed with dry  $\text{CH}_2\text{Cl}_2$  (5x10 ml).

25 Example 45      Cleavage of a Library of Di-, Tri- and Tetrasaccharides from the Resin Affording Glycosyl Amine of Oligosaccharides  
(Figure 12)

45 A mixture of mono-, di- and tri-O-(2,3,4-tri-O-benzyl  $\alpha,\beta$ -L-fucopyranosyl) (1 $\rightarrow$ 2), (1 $\rightarrow$ 3), (1 $\rightarrow$ 4), (1 $\rightarrow$ 6)- $\beta$ -  
30 D-glucopyranosyl amine

The resin from Example 44 was treated with  $\text{NH}_3/\text{MeOH}$  (10 ml) for 5 min. The resin was filtered off, and the filtrate was evaporated giving a mixture of disaccharides, trisaccharides, and tetrasaccharides.

35 FAB MS disaccharides  $\text{C}_{33}\text{H}_{41}\text{NO}_9$  (595.66), trisaccharides  $\text{C}_{60}\text{H}_{69}\text{NO}_{13}$  (1012.16), tetrasaccharides  $\text{C}_{87}\text{H}_{97}\text{NO}_{17}$  (1429.66)

(m/z (%)) 618 [M<sub>di</sub>+Na]<sup>+</sup> (41), 596 [M<sub>di</sub>+H]<sup>+</sup> (57), 1034 [M<sub>tri</sub>+Na]<sup>+</sup> (56), 1012 [M<sub>tri</sub>+H]<sup>+</sup> (100), 1450 [M<sub>tetra</sub>+Na]<sup>+</sup> (8), 1428 [M<sub>tetra</sub>+H]<sup>+</sup> (10).

5    Example 46            Synthesis of a Second Sugar - Glycosyl  
                                  Amine - Ddh Linker - Resin Conjugate  
                                  (Figure 13)

46 O-(2,3,6-tri-O-benzyl-4-O-bromoacetyl- $\alpha,\beta$ -D-galactopyranosyl) (1 $\rightarrow$ 4) - [N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl)] 2,3,6-tri-O-benzyl- $\beta$ -D-galactopyranosyl amine - MBHA resin conjugate

15 A mixture of resin from Example 39 and ethyl 2,3,6-tri-O-benzyl-4-O-bromoacetyl-1-thio- $\beta$ -D-galactopyranoside (1.25 g, 2 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (10 ml) was treated with dimethyl(methylthio)sulphonium trifluoromethanesulphonate (DMTST) (1.50 g, 5.81 mmol) at room temperature for 1 h. The resin was washed with dry  $\text{CH}_2\text{Cl}_2$  (5x10 ml).

20

Example 47                      Selective Deprotection of the Second Sugar  
- Glycosyl Amine - Ddh Linker - Resin  
Conjugate (Figure 13)

47 O-(2,3,6-tri-O-benzyl- $\alpha,\beta$ -D-galacto-pyranosyl) (1 $\rightarrow$ 4) -  
25 [N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene) -  
hexanoic acid-6-yl)] 2,3,6-tri-O-benzyl- $\beta$ -D-  
galactopyranosyl amine - MBHA resin conjugate

The resin from Example 46 was gently agitated with sodium methoxide (200 mg, 3.70 mmol) in abs. MeOH (5 ml) at room temperature for 1 h. The resin was washed with abs. MeOH (5x10 ml), DMF(5x10 ml), ether (5x10 ml) and dried under high vacuum for 1 h, giving the resin bonded partially unprotected disaccharide. A sample of resin (5 mg) was cleaved by NH<sub>3</sub>/MeOH (Example 41) and the resulting product was analyzed by TLC and mass spectrometry, proving the quantitative deprotection.

- 40 -

Example 48      Cleavage of a Second Sugar - Glycosyl Amine  
- Ddh Linker - Resin Conjugate Affording a  
Glycosyl Amine of a Disaccharide  
(Figure 13)

5    48    *O*-(2,3,6-tri-*O*-benzyl- $\alpha,\beta$ -D-galacto-pyranosyl) (1 $\rightarrow$ 4) -  
2,3,6-tri-*O*-benzyl- $\beta$ -D-galactopyranosyl amine

The resin from Example 47 was treated with  
NH<sub>3</sub>/MeOH (10 ml) for 5 min. The resin was filtered off, and  
the filtrate was evaporated giving an anomeric mixture of  
10    disaccharides. FAB MS C<sub>54</sub>H<sub>59</sub>NO<sub>10</sub> (882.01) (m/z (%)) 904  
[M+Na]<sup>+</sup> (100), 880 [M+H]<sup>+</sup> (41).

Example 49      Cleavage of a Carbohydrate - Ddh-  
Aminobenzyl Linker - Resin Conjugate  
15      Affording an Aminobenzyl Protected  
Carbohydrate (Figure 14)

49    4-aminobenzyl  $\beta$ -D-glucopyranoside

The resin from Example 29 was treated with  
NH<sub>3</sub>/MeOH (5 ml) overnight. The resin was filtered off, and  
20    the filtrate was evaporated giving 4-aminobenzyl  $\beta$ -D-  
glucopyranoside.

R<sub>f</sub> 0.55 (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:4:0.5 v/v/v); FAB MS C<sub>11</sub>H<sub>19</sub>NO<sub>5</sub>  
(269.28) m/z (%) 402 [M+Cs]<sup>+</sup> (25), 292 [M+Na]<sup>+</sup> (50), 270  
25    [M+H]<sup>+</sup> (18).

Example 50      Deprotection of 4-Aminobenzyl Protected  
Carbohydrate (Figure 14)

50     $\beta$ -D-Glucopyranose

30      4-Aminobenzyl  $\beta$ -D-glucopyranoside (110 mg,  
0.40 mmol) was hydrogenated over Pd/C (10%) (50 mg) in MeOH  
(5 ml) at room temperature for 4 hours. The catalyst was  
filtered off and the filtrate was evaporated affording  
D-glucose in quantitative yield.

35

Example 51                    Immobilization of an Oligosaccharide  
(Figure 15)

51     O-[O-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-  
glucopyranosyl(1 $\rightarrow$ 4))-2,3,6-tri-O-acetyl- $\beta$ -D-  
5     glucopyranosyl(1 $\rightarrow$ 4)]-2,3,6-tri-O-acetyl- $\beta$ -D-  
glucopyranosyl amine using 6-hydroxy-6-(4,4-dimethyl-  
2,6-dioxocyclohexylidene)-hexanoic acid - MBHA resin  
conjugate

10     The resin from Example 37 was reacted with O-[O-  
(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 4))-2,3,6-  
tri-O-acetyl- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 4)]-2,3,6-tri-O-acetyl-  
 $\beta$ -D-glucopyranosyl amine (1.80 g, 2.00 mmol) in DMF (5 ml)  
at room temperature for 2 days. The resin was washed with  
DMF (5x10 ml).

15

Example 52                    Synthesis of an aminosugar - Ddh - resin  
conjugate (Figure 16)

52     N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic  
acid-6-yl) D-glucosamine - MBHA resin conjugate  
20     A mixture of resin from Example 37 and  
glucosamine (350 mg, 2 mmol) in DMF (20 ml) was stirred at  
room temperature for 2 days. The resin was filtered off,  
washed with DMF/H<sub>2</sub>O 4:1 (5x10 ml), DMF 5x10 ml, MeOH  
(5x10), ether (5x10 ml), and dried under high vacuum  
25     overnight.

30     It will be apparent to the person skilled in the  
art that while the invention has been described in some  
detail for the purposes of clarity and understanding,  
various modifications and alterations to the embodiments  
and methods described herein may be made without departing  
from the scope of the inventive concept disclosed in this  
invention.

35     References cited herein are listed on the  
following pages, and are incorporated by this reference.

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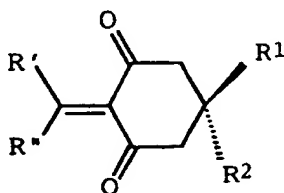
CLAIMS

1. A support for solid-phase synthesis of oligosaccharides, said support comprising

- 5 a) a resin,  
b) a linker covalently attached to the resin,  
and

c) one or more saccharide groups covalently attached to the resin via the linker,

wherein the linker is a 2-substituted-1,3-dioxocycloalkane compound, and  
10 said support having general formula I



I

15 in which

R<sup>1</sup> and R<sup>2</sup> may be the same or different, and is each hydrogen or C<sub>1-4</sub> alkyl; preferably both R<sup>1</sup> and R<sup>2</sup> are  
20 methyl;

R' is an amino sugar, a glycosylamine, or a glycosylamine of an oligosaccharide; a mono or oligosaccharide coupled through an alkyl-, substituted alkyl-, aryl-, substituted aryl-, cycloalkyl-, or  
25 substituted cycloalkyl-amino group; or a mono or oligosaccharide coupled through a carboxyalkyl-, substituted carboxyalkyl-, carboxyaryl-, substituted carboxyaryl-, carboxycycloalkyl-, or substituted carboxycycloalkyl-amino group, and

30 R'' is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group which is directly coupled to the resin support, or which may optionally be coupled to the resin

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support via a covalent linkage which is stable to conditions of oligosaccharide synthesis and cleavage.

2. A support according to Claim 1, in which both R<sup>1</sup> and R<sup>2</sup> are methyl.

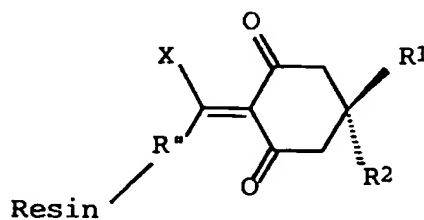
5 3. A support according to Claim 1 or Claim 2, in which R<sup>1</sup> is an oligosaccharide-O-CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)-NH, monosaccharide-O-CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)-NH, amino-oligosaccharide-CO<sub>2</sub>CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)NH, or amino-monosaccharide-CO<sub>2</sub>CH<sub>2</sub>-(C<sub>6</sub>H<sub>4</sub>)-NH group.

10 4. A support according to any one of Claims 1 to 3, in which the covalent linkage to the resin is provided by a -CONH-, -O-, -S-, -COO-, -CH=N-, -NHCONH-, -NHCSNH, or -NHNH- grouping.

5. A support according to any one of Claims 1 to 4, 15 in which the linker is functionalised Dde, Ddh or ODMab.

6. A support according to any one of Claims 1 to 5, comprising a resin, a linker and a monosaccharide, an oligosaccharide, an aminosaccharide or an amino-oligosaccharide.

20 7. A support for solid-phase synthesis comprising a resin and a linker group, wherein the linker is a 2-substituted-1,3-dioxocycloalkane of general formula II:



II

in which

X is OH or NH<sub>2</sub>;

30 R<sup>1</sup> and R<sup>2</sup> may be the same or different, and is each hydrogen or C<sub>1-4</sub> alkyl; and

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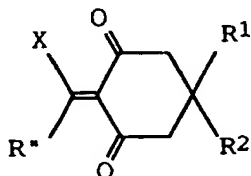
R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group which is directly coupled to the resin support; or which may optionally be coupled to the resin support via a covalent linkage which is stable to conditions of oligosaccharide synthesis and cleavage.

8. A support according to Claim 7, in which R<sup>1</sup> and R<sup>2</sup> are both methyl

9. A support according to Claim 7 or Claim 8, in which the covalent linkage to the resin is provided by a -CONH-, -O-, -S-, -COO-, -CH=N-, -NHCONH-, -NHCSNH, or -NHNH- grouping.

10. A linker-saccharide complex in which the linker group is as defined in Claim 1 or Claim 2 and the saccharide is as defined in Claim 1 or Claim 6.

11. A compound carrying functional groups suitable to attach a primary amine to a resin via covalent bonds which are stable to conditions of oligosaccharide synthesis and cleavage, said compound having general formula III



III

in which

X is OH or NH<sub>2</sub>;

25 R<sup>1</sup> and R<sup>2</sup> may be the same or different, and is each hydrogen or C<sub>1-4</sub> alkyl, and

R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group, which carries a functionality capable of reacting with a functionalised resin.

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12. A compound according to Claim 11, in which both  $R^1$  and  $R^2$  are methyl.
13. A compound according to Claim 11 or Claim 12, in which the functionality on R" is a carboxyl group.
- 5 14. A compound according to Claim 11, which is 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid or an ester thereof.
15. A compound according to Claim 14, in which the ester is a benzyl, methyl or t-butyl ester.
- 10 16. A support according to any one of Claims 1 to 6, in which the linker is a compound according to any one of Claims 11 to 15.
17. A support according to any one of Claims 7 to 9, in which the linker is a compound according to any one of
- 15 Claims 11 to 15.
18. A linker-saccharide complex according to Claim 10, in which the linker is a compound according to any one of Claims 11 to 15.
19. A kit for solid phase synthesis or combinatorial
- 20 synthesis of oligosaccharides, comprising:
- a) a resin-linker-saccharide support according to any one of Claims 1 to 5 or 16,
- b) a linker-saccharide complex according to Claims 10 or 17, or
- 25 c) a resin-linker support according to any one of Claims 7 to 17,
- and optionally also comprising one or more protecting agents, deprotecting agents, and/or solvents suitable for solid phase or combinatorial synthesis.
- 30 20. A method of solid-phase synthesis of oligosaccharides, comprising the step of sequentially linking mono- or oligosaccharide groups to a support as defined in any one of Claims 1 to 9 or 16.
21. A method of synthesis of a linker group according
- 35 to general formula I as defined in Claim 1, comprising the step of C-acylation of a 2-substituted 1,3-dioxocyclohexane compound with a dicarboxylic acid, and

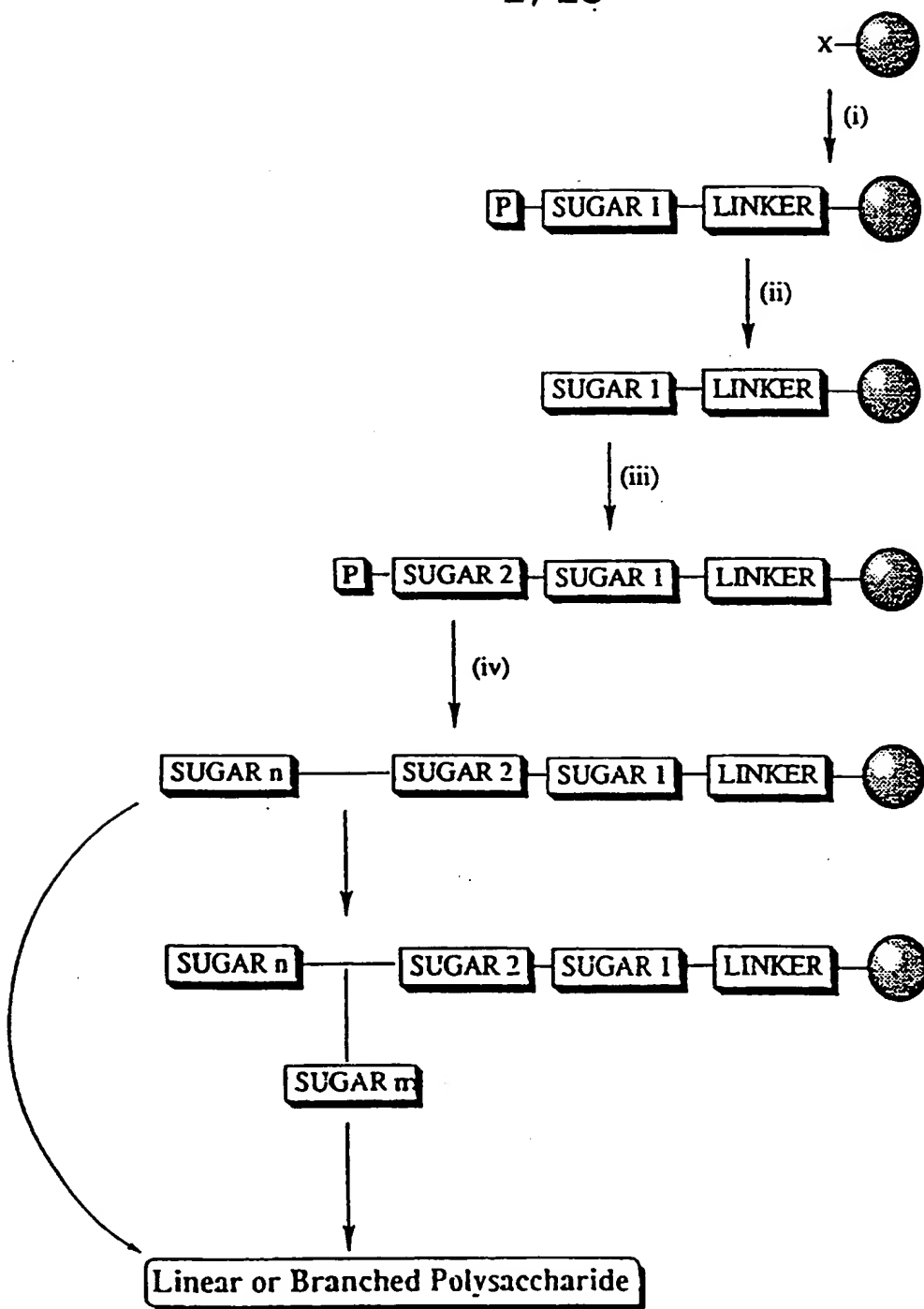
- 48 -

optionally reacting the product of the C-acylation reaction with 4-aminobenzyl alcohol, to form the 4-aminobenzyl derivative.

22. A method according to Claim 21, in which the  
5 dicarboxylic acid is mono-protected by ester formation.
23. A method according to Claim 21 or Claim 22, in which the C-acylation reaction is activated with carbodiimide and catalysed by N,N'-dimethylaminopyridine.
24. A method of synthesis of a resin-linker support  
10 according to any one of Claims 6 to 9, comprising the step of swelling a resin in a suitable solvent, treating the swollen resin with a dicarboxylic acid, and reacting the thus-produced product with a 2-substituted 1,3-dioxocycloalkane compound.
- 15 25. A method according to any one of Claims 21 to 24, in which the 2-substituted 1,3-dioxocycloalkane compound is 5,5-dimethyl-1,3-cyclohexanedione.
26. A method according to any one of Claims 21 to 25, in which the dicarboxylic acid is adipic acid.

20

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**Conditions:** (i) Attachment of sugar-linker conjugate to a resin support.  
(ii) Selective deprotection of one sugar hydroxyl group.  
(iii) Coupling of next sugar residue.  
(iv) Repeat of steps (ii) and (iii) as desired.

FIGURE 1

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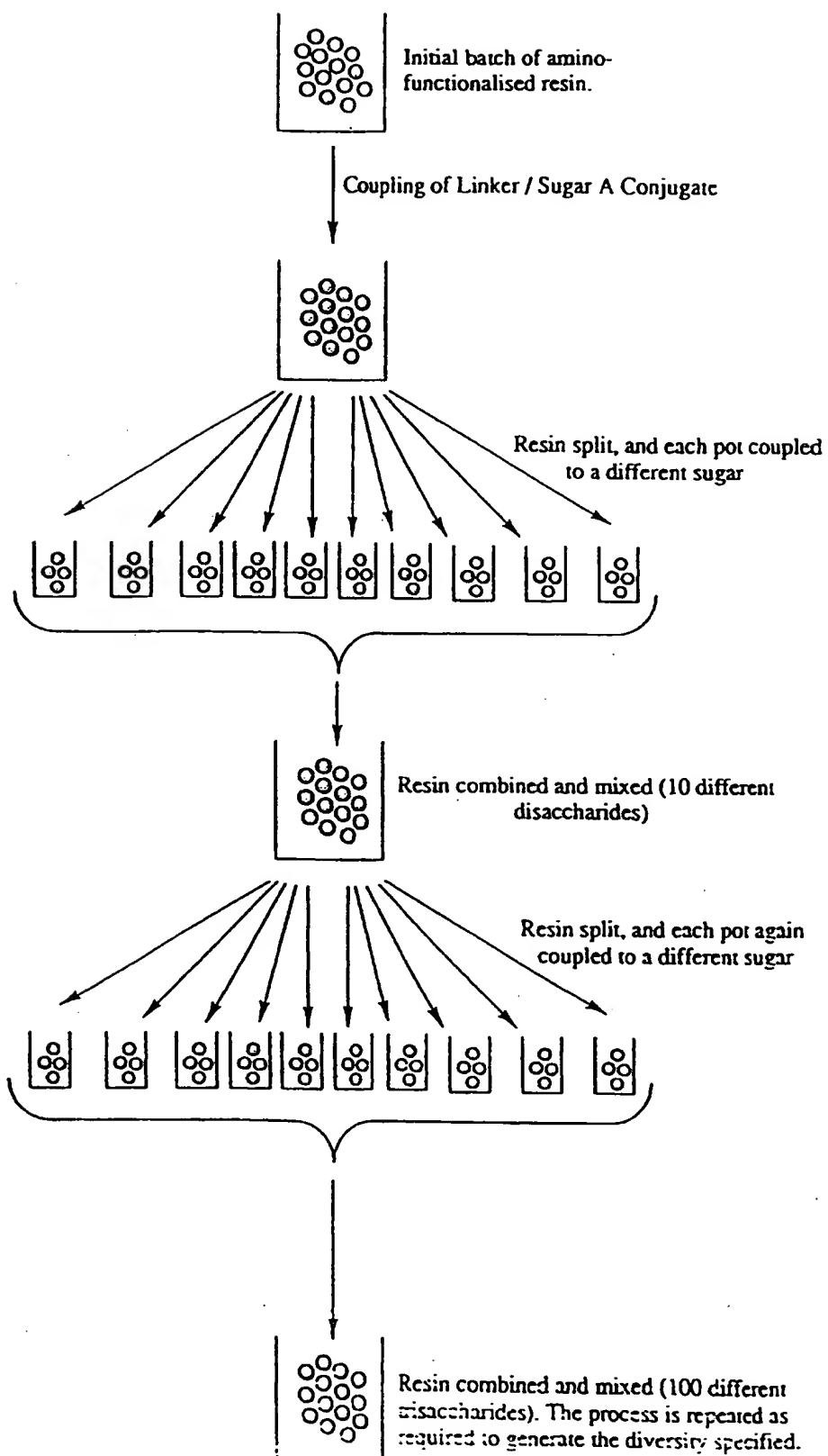


FIGURE 2

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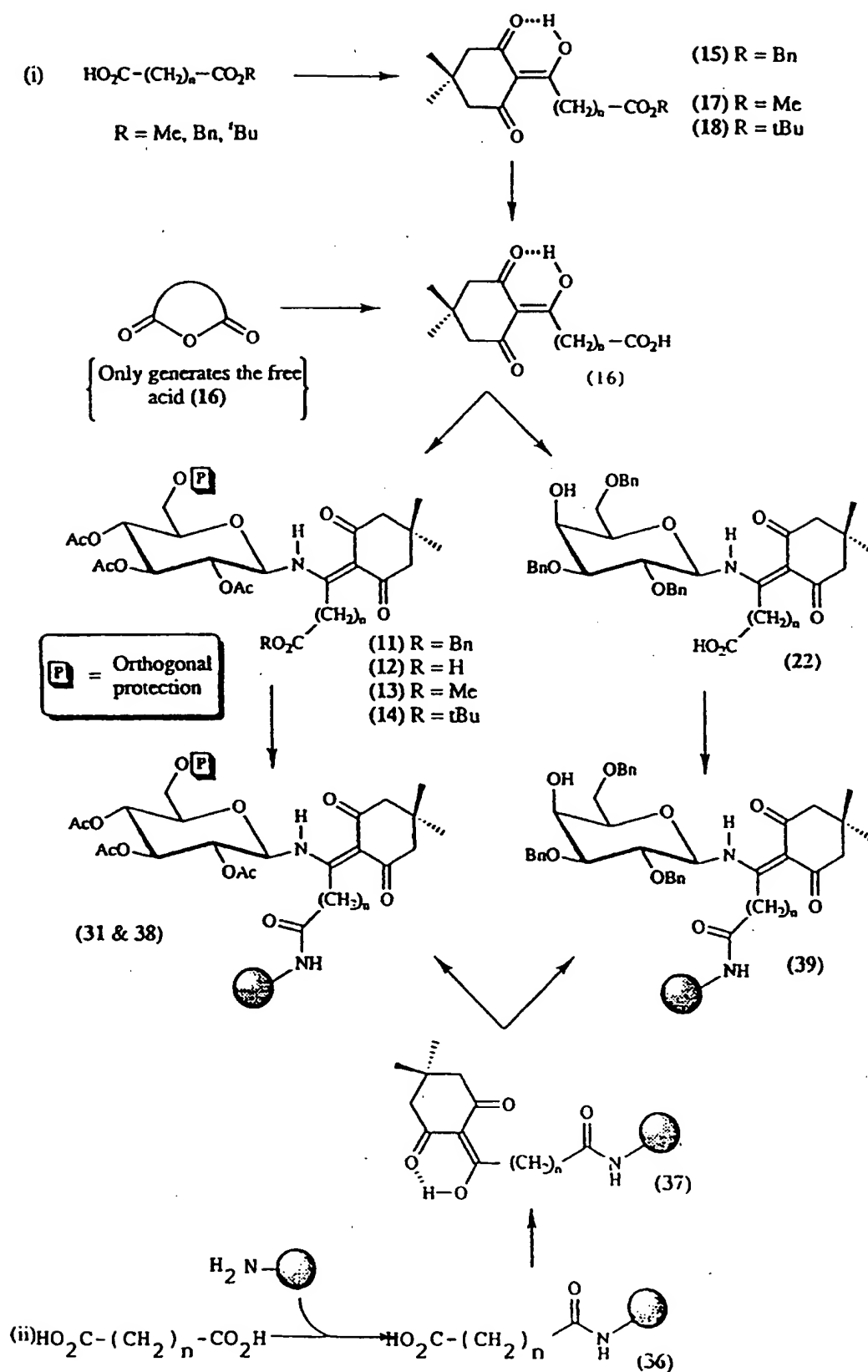


FIGURE 3

SUBSTITUTE SHEET (Rule 26)

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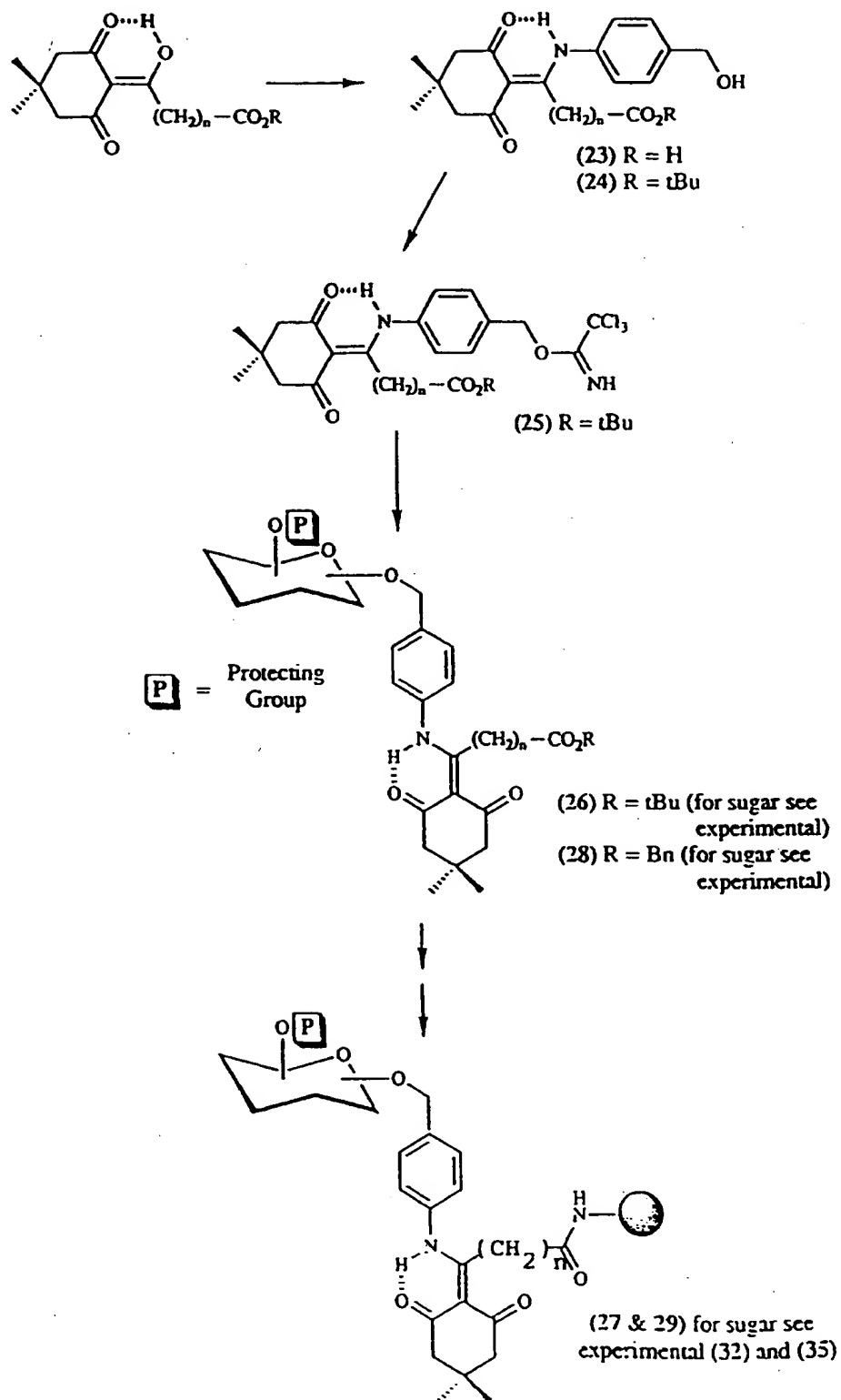
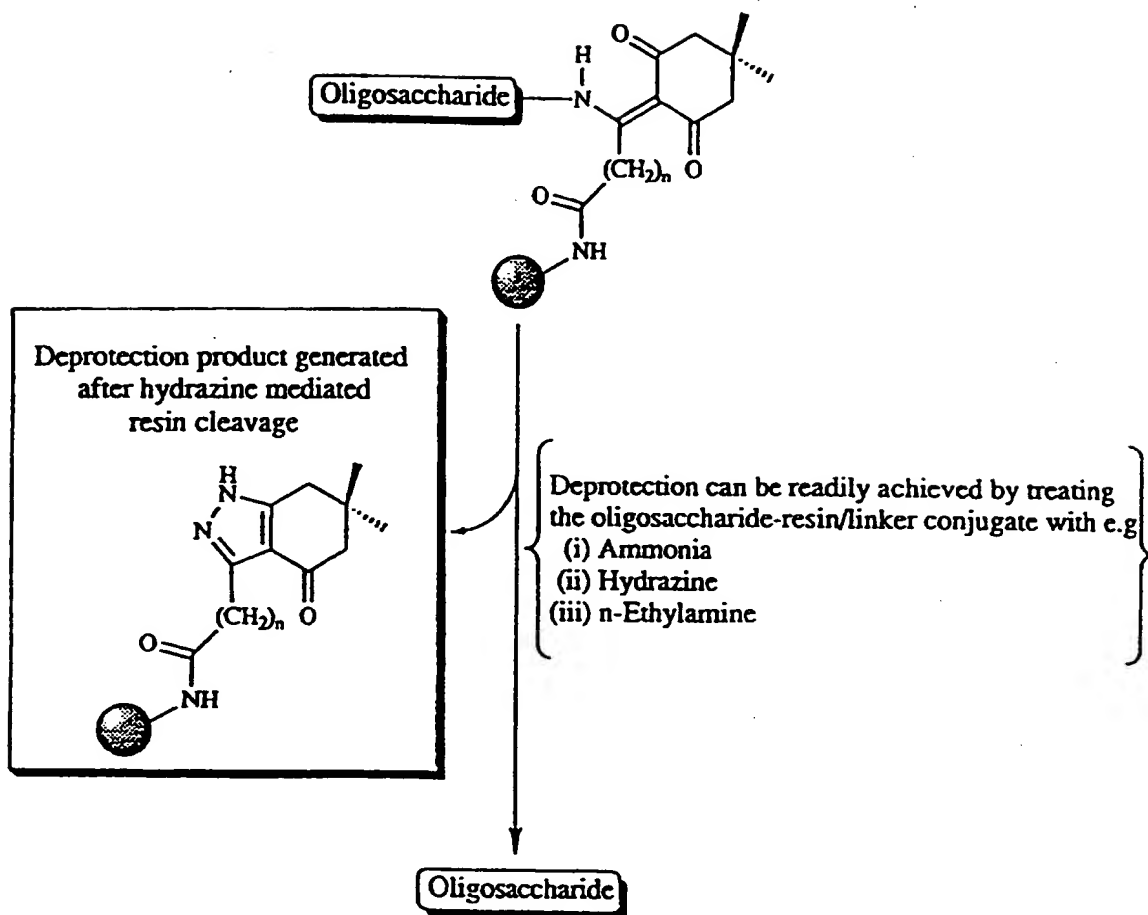


FIGURE 4

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NB. The Oligosaccharide can potentially be released in either the protected or deprotected form depending on the choice of monomer protection employed during the synthesis.

FIGURE 5

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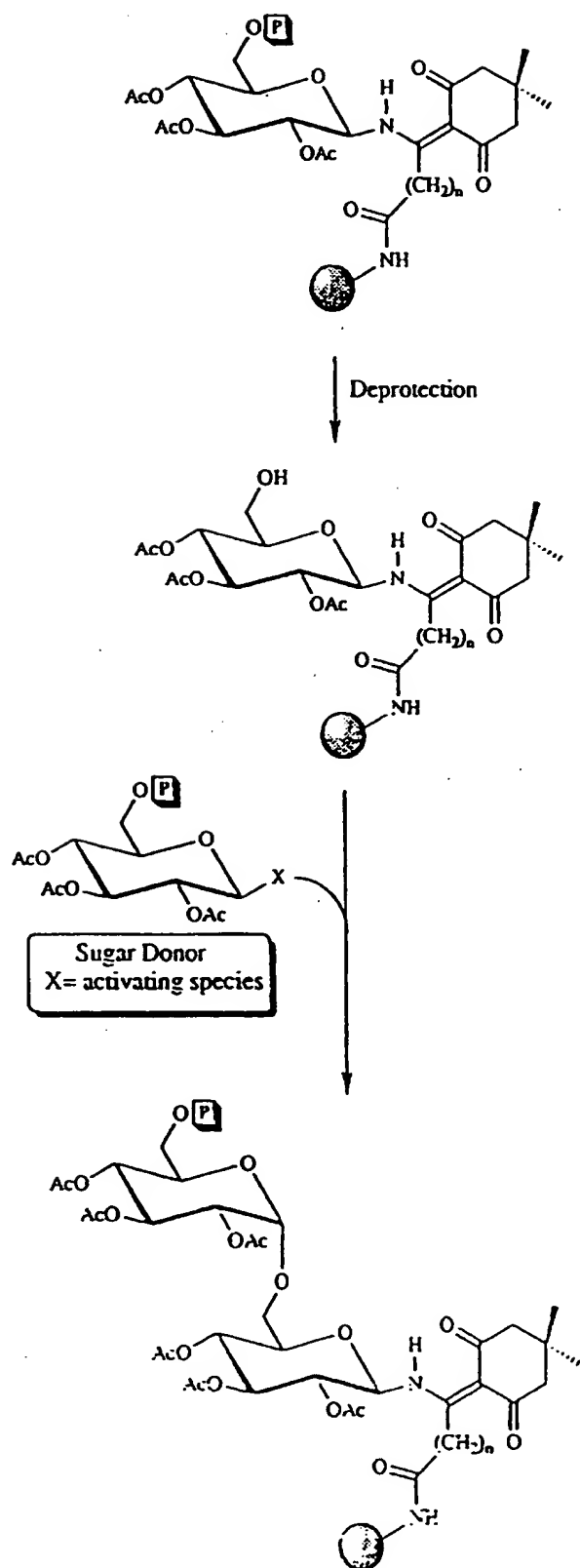


FIGURE 6

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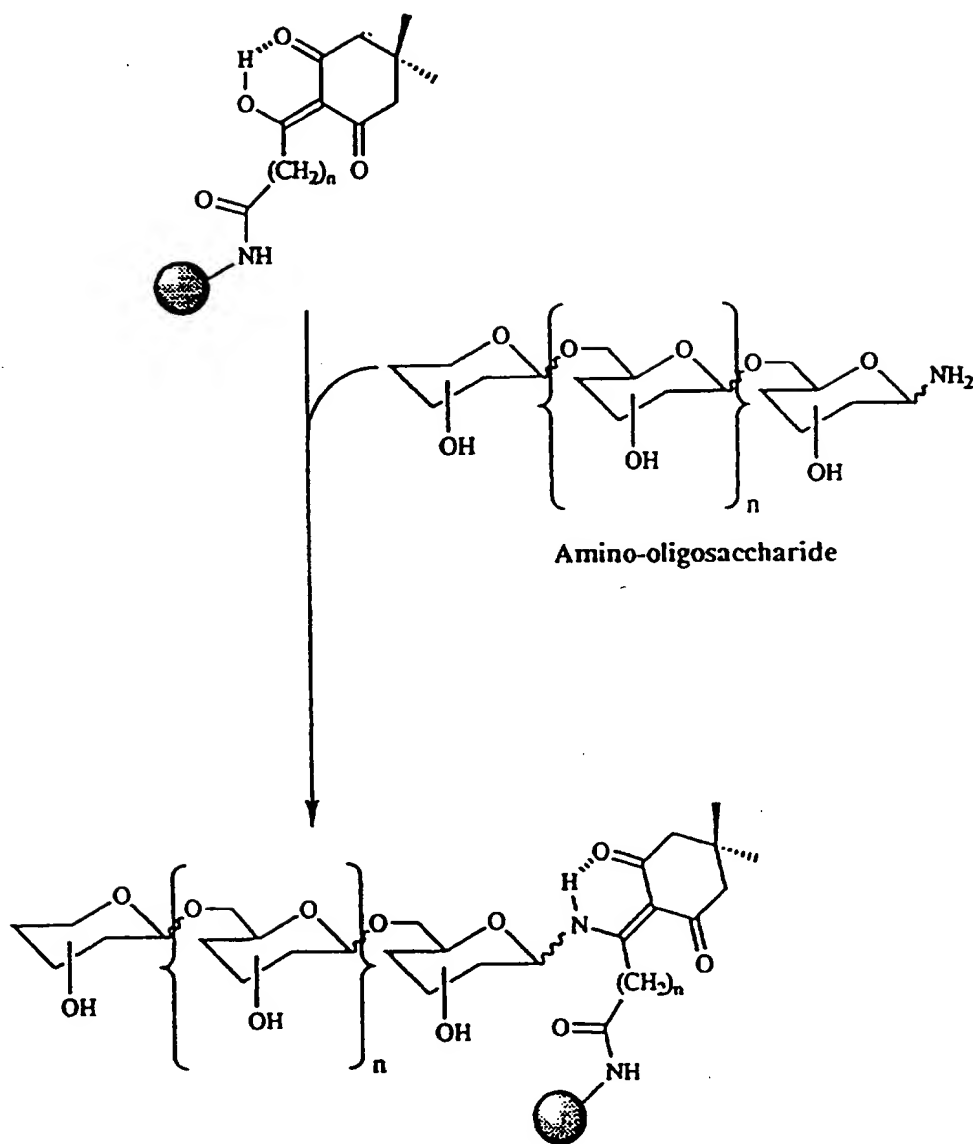


FIGURE 7

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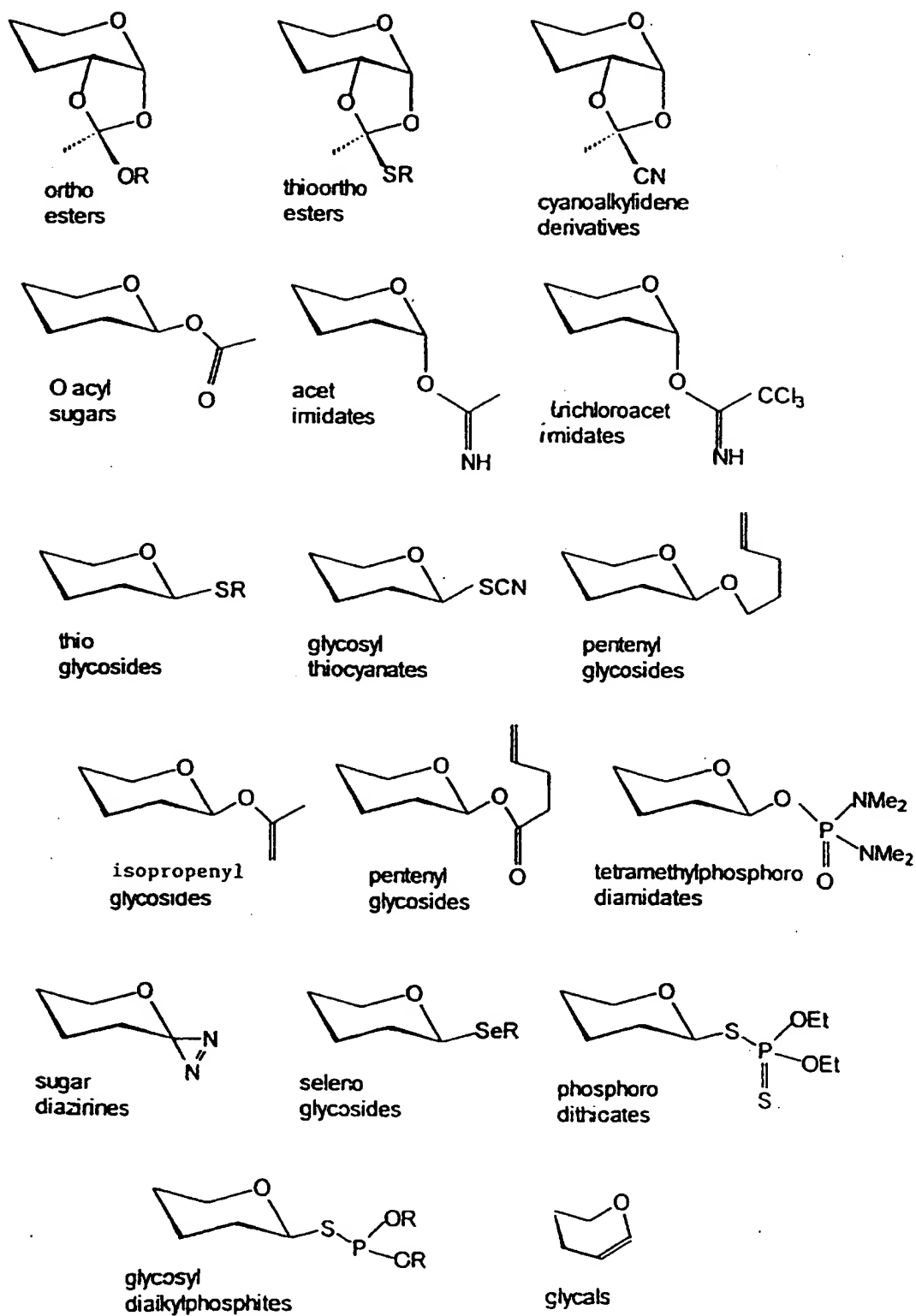


FIGURE 8

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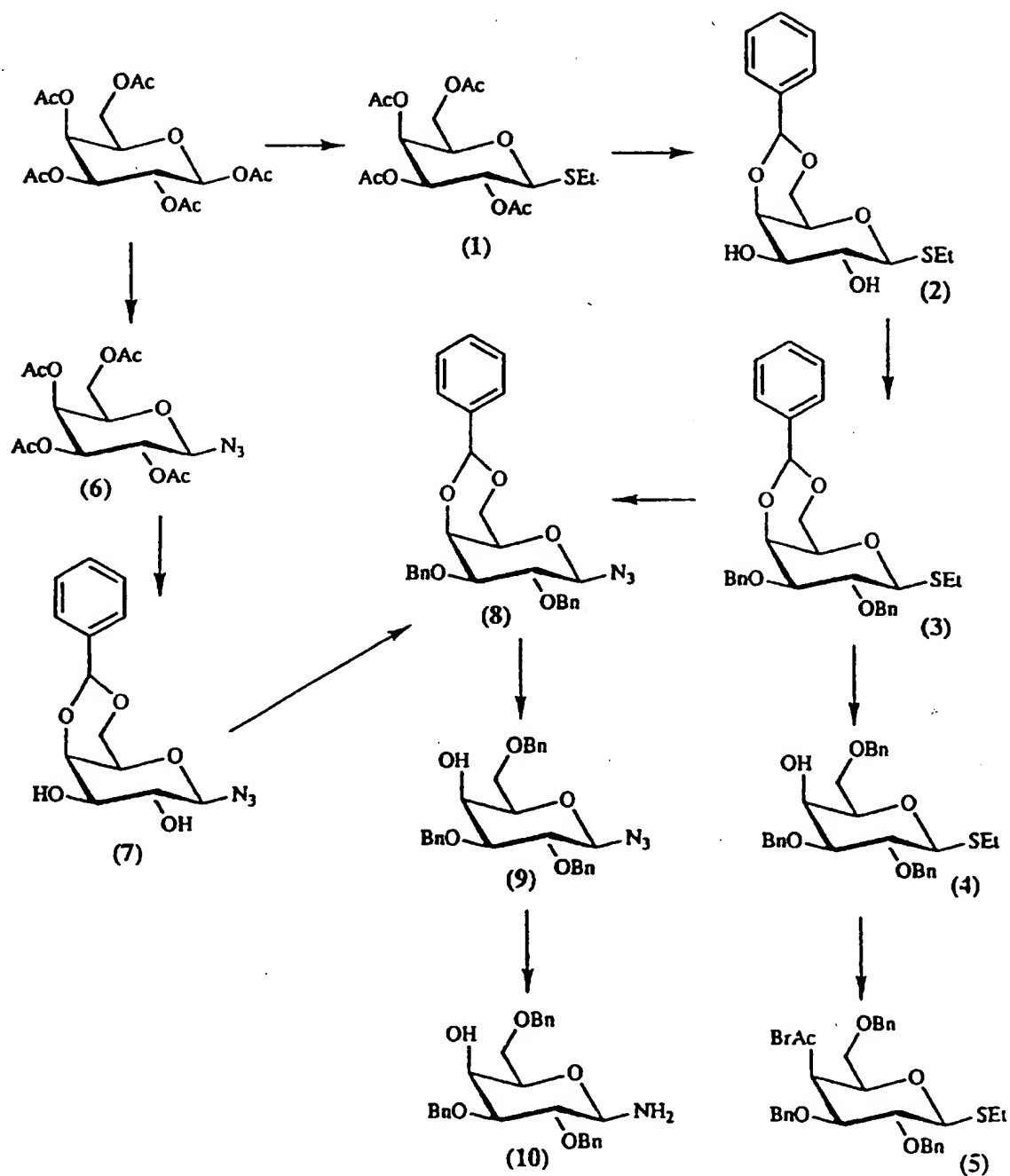


FIGURE 9

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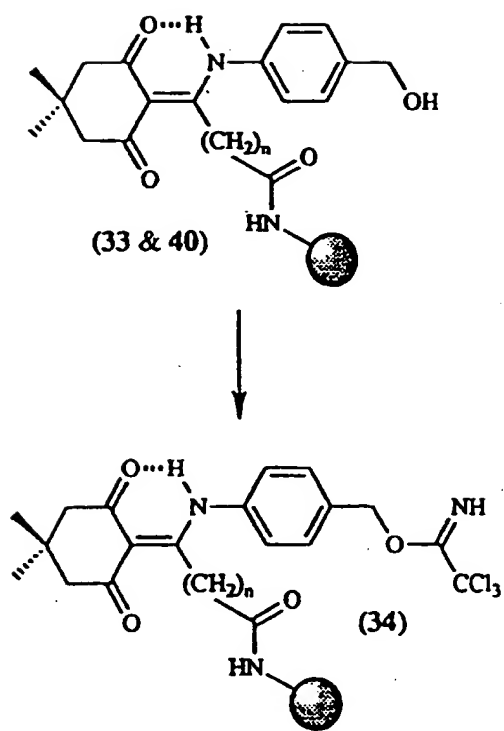


FIGURE 10

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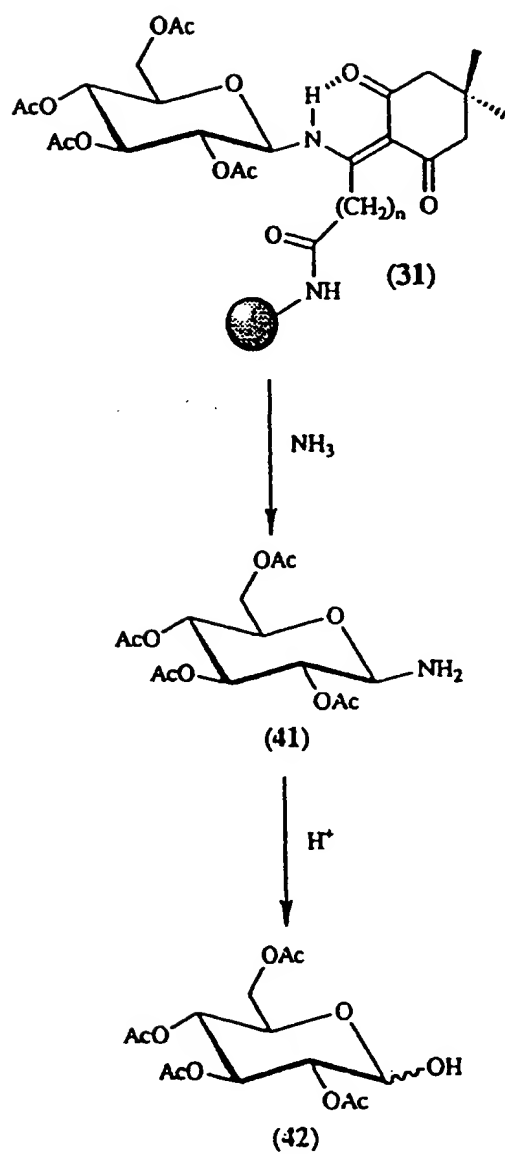


FIGURE 11

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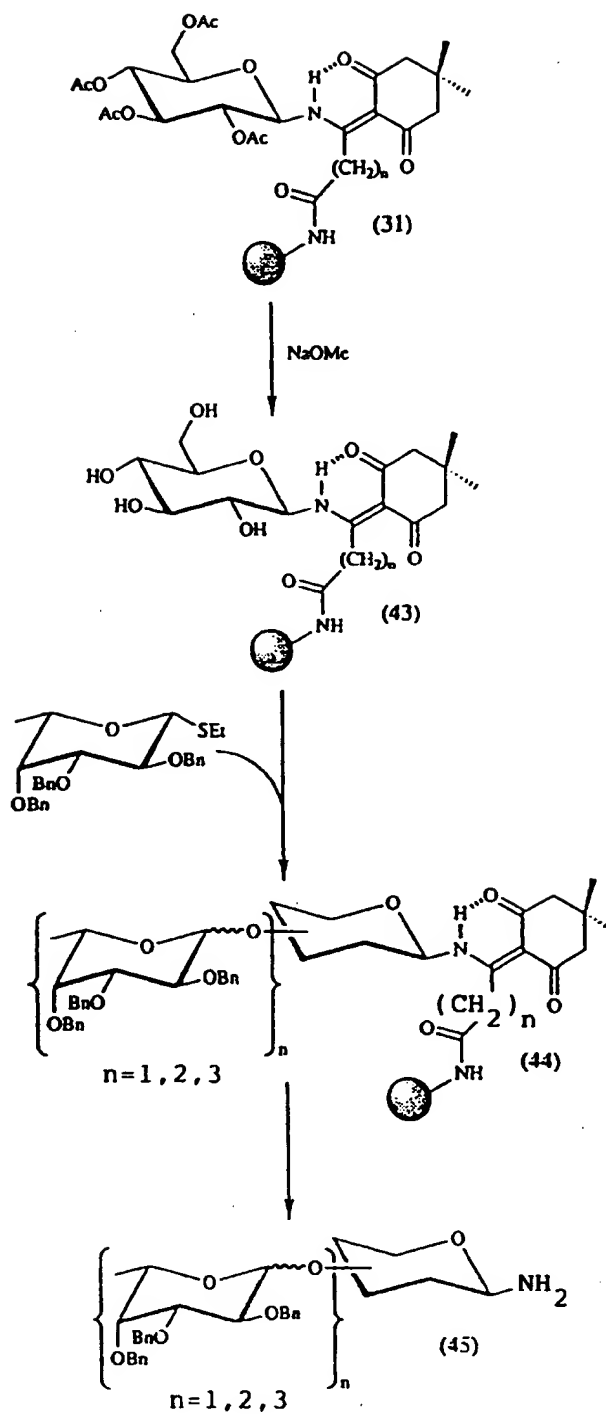


FIGURE 12

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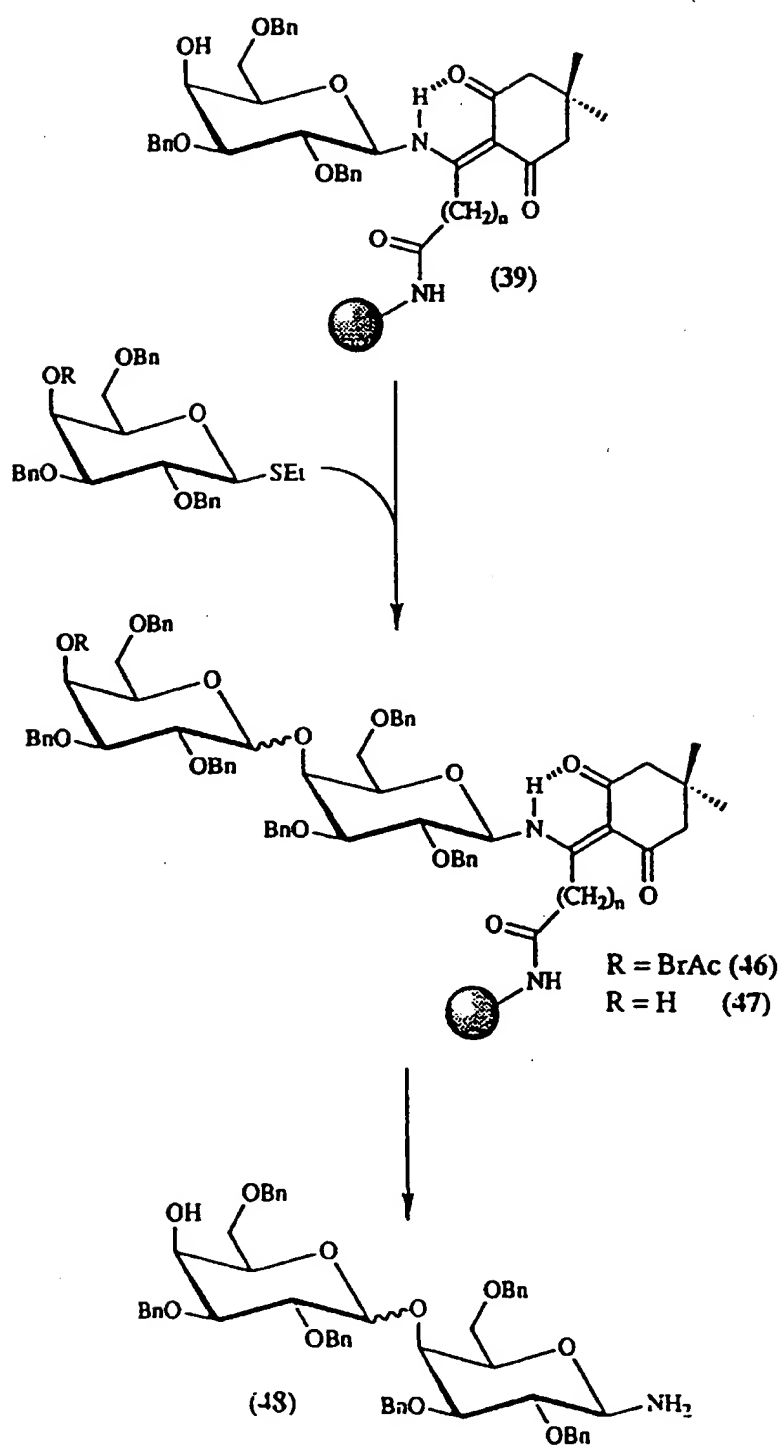


FIGURE 13

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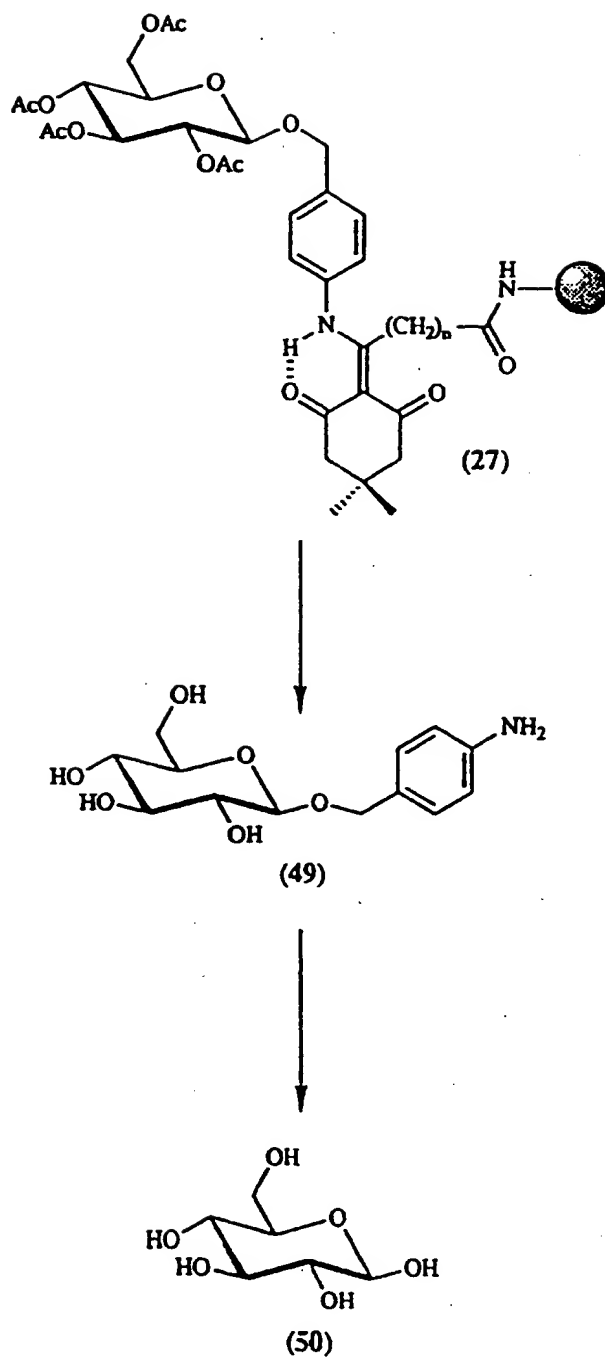


FIGURE 14

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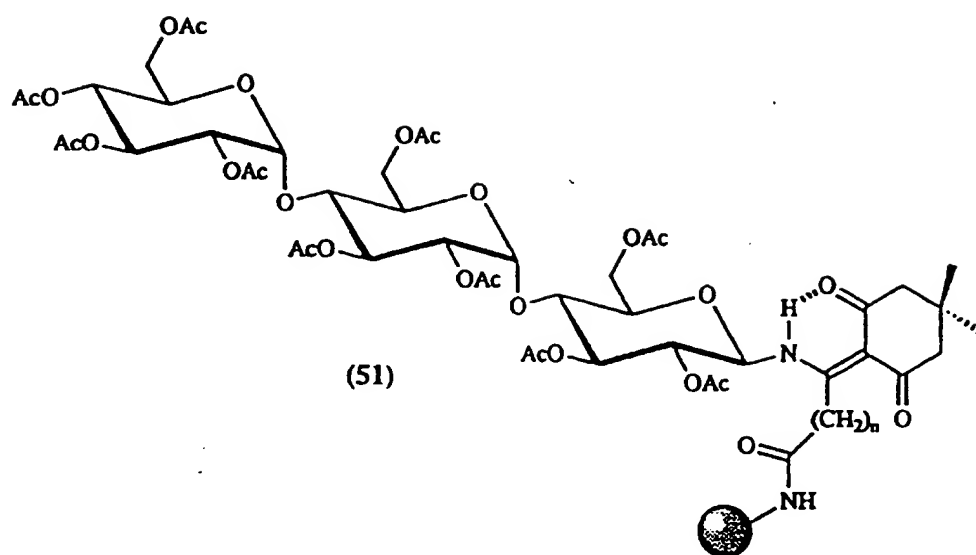


FIGURE 15

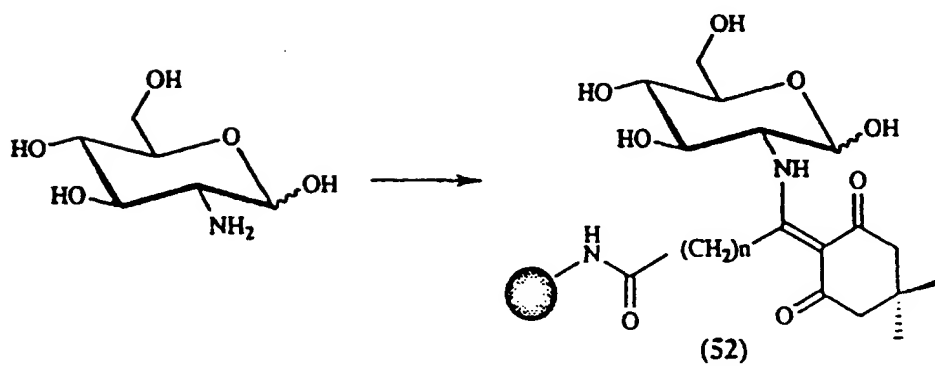


FIGURE 16

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00544

**A. CLASSIFICATION OF SUBJECT MATTER**Int Cl<sup>6</sup>: C07C 59/353, 59/90, 69/716, 69/738, 229/32, 257/06, C07H 5/06, C08J 7/14, 7/16

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
CHEMICAL ABSTRACTS, Substructure Search.**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Bioorganic and Med. Chem. Lett., 1996, 6(13), 1525-1528, W. Bannwarth et al., "A New Linker for Primary Amines applicable to Combinatorial approaches". Cited in the application.	1-26
A	Tetrahedron, 1996, 52(4), 1095-1121, G-J. Boons, "Strategies in Oligosaccharide Synthesis". See in particular pages 1113-1116	1-26
A	I.A. Nash et al., Tetrahedron Letters, 1996, 37(15), 2625-2628, "Dde-A selective Primary amine Protecting Group: A Facile Solid Phase Synthetic Approach to Polyamine Conjugates".	1-26

☐ Further documents are listed in the continuation of Box C☐ See patent family annex

## \* Special categories of cited documents:

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Date of the actual completion of the international search  
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Date of mailing of the international search report

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